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The Effect of (−)-Epigallo-catechin-(3)-gallate on Amyloidogenic Proteins Suggests a Common Mechanism

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Abstract

Studies on the interaction of the green tea polyphenol (−)-Epigallocatechin-3-gallate (EGCG) with fourteen disease-related amyloid polypeptides and prions Huntingtin, Amyloid-beta, alpha-Synuclein, islet amyloid polypeptide (IAPP), Sup35, NM25 and NM4, tau, MSP2, semen-derived enhancer of virus infection (SEVI), immunoglobulin light chains, beta-microglobulin, prion protein (PrP) and Insulin, have yielded a variety of experimental observations. Here, we analyze whether these observations could be explained by a common mechanism and give a broad overview of the published experimental data on the actions of EGCG. Firstly, we look at the influence of EGCG on aggregate toxicity, morphology, seeding competence, stability and conformational changes. Secondly, we screened publications elucidating the biochemical mechanism of EGCG intervention, notably the effect of EGCG on aggregation kinetics, oligomeric aggregation intermediates, and its binding mode to polypeptides. We hypothesize that the experimental results may be reconciled in a common mechanism, in which EGCG binds to cross-beta sheet aggregation intermediates. The relative position of these species in the energy profile of the amyloid cascade would determine the net effect of EGCG on aggregation and disaggregation of amyloid fibrils.

Keywords

Epigallocathechin-3-gallate (EGCG); Amyloid polypeptides; Aggregation; Fibrils

7.1 Introduction

The polyphenol (−)-Epigallocatechin-3-gallate (EGCG) is produced by the tea plant (*Camellia sinensis*) and accounts for about 10 % of the dry weight of its leaves (Graham 1992). The flavonoid EGCG belongs to the subclass of flavan-3-ols which have an unsaturated C-ring with the B-ring attached to their C2-atom (Fig. 7.1a). In EGCG, the B-ring is attached in epi conformation and the 3-ol of the C-ring is substituted by an 3-O-gallate function (Beecher 2003). EGCG has been found to inhibit formation of amyloid

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fibrils and to bind to existing amyloid fibrils and remodel them into non-amyloid aggregates (Fig. 7.1b) (Ehrnhoefer et al. 2006, 2008; Bieschke et al. 2010). Its anti-aggregation mechanism has since been studied for 14 amyloidogenic peptides and proteins. At first glance, the data seem to suggest that EGCG has quite different effects on the different polypeptides.

Our review aims to compare these effects on a phenomenological and mechanistic level. To do so, we will concentrate on disease-related polypeptides and prions. In the first part, we will give a broad overview of the published experimental data on the action of EGCG, while in the second part we will discuss how the experimental results might be reconciled in a general mechanism that leads to the inhibition of amyloidogenic aggregation and the disaggregation of amyloid fibrils.

7.2 Phenomenological Overview of EGCG

About 10 years ago, it was first reported that green tea extract and EGCG reduced the cytotoxicity of the Amyloid-beta peptide (Aβ) involved in Alzheimer's Disease (AD) (Levites et al. 2003) and also reduced the amount and size of amyloid deposits in APP transgenic mice (Rezai-Zadeh et al. 2005). Likewise, it diminished the formation of toxic aggregates in yeast and *Drosophila* models of Huntingtin protein (Htt) aggregation (Ehrnhoefer et al. 2006). These early results prompted a large number of studies on the effect of EGCG on various amyloidogenic proteins. Tables 7.1, 7.2, and 7.3 present an overview of the effects of EGCG extracted from data that were published prior to August 2014. Empty fields mark characteristics for which no data are available for the respective polypeptide. Some characteristics have been addressed in an extensive number of studies; in these cases, the complete reference list is included in the overview table while selected studies are referenced in the text.

7.2.1 Amyloid Toxicity

Amyloid fibrils may have functional roles (Chapman et al. 2002; Berson et al. 2003; Fowler et al. 2006). Several functional prions that possess amyloid structural elements have been identified in yeast and other fungi (Shorter and Lindquist 2005; Coustou et al. 1997). However, in many polypeptides amyloid formation is toxic to cells. Indeed, deposition of misfolded polypeptides in amyloid or amyloid-like aggregates is characteristic of some of the most prominent neurodegenerative diseases: the Amyloid-beta peptides (Aβ) derived from the Amyloid Precursor Protein (APP) and the tau protein in Alzheimer's disease (AD) (Golde et al. 1993; Kosik et al. 1986), Huntingtin protein (Htt) in Huntington's disease (HD) (Trottier et al. 1995; Scherzinger et al. 1997), alpha-Synuclein (α-Syn) in Parkinson's disease (PD) (Takeda et al. 1998), and the prion protein PrP in transmissible spongiform encephalopathies (TSE) (Prusiner 1998). Amyloid deposits also form in systemic diseases: globular monoclonal immunoglobulin light chains (LC) and Transthyretin (TTR) deposits form in the heart and in other tissues in systemic Light Chain Amyloidosis (AL) (Solomon et al. 1982) and in Transthyretin-Amyloidosis, respectively (Saraiva et al. 1984). In Diabetes Mellitus type II (DM II) the Islet Amyloid Polypeptide (IAPP, amylin) forms amyloid deposits in the insulin producing islet-β-cells (Johnson et al. 1989). *In vitro*, it was observed

that also insulin is capable of forming amyloid fibrils under physiological conditions, albeit at high concentrations of 345 μM (Wang et al. 2012a). Diabetes mellitus has large economic implications; about 347 million people worldwide suffer from DM type I and type II (Danaei et al. 2011), which dwarfs even prevalence of AD (estimated 44 million cases in 2013) (Prince et al. 2014).

Patients undergoing long-term hemodialysis are at risk of developing dialysis-related amyloidosis (DRA), where β_2-Microglobin (β2m) is deposited, a light chain of the major histocompatibility complex I (MHC-I) (Grey et al. 1973) that is prone to form amyloid deposits (Gejyo et al. 1985, Linke 1985).

Interestingly, two other devastating diseases are also indirectly connected to amyloid formation. An amyloid forming-protein derived from human semen was found to enhance the infectious potential of HIV and was therefore named semen-derived enhancer of virus infection (SEVI). The parasite plasmodium falciparum involved in malaria pathogenesis produces a highly abundant surface protein, the merozoite surface protein 2 (MSP2) which is considered as a candidate for a malaria vaccine (Genton et al. 2002). It was shown that MSP2 has also an amyloidogenic nature (Yang et al. 2007). While this is by no means a complete list of amyloid diseases, experimental data on EGCG have been collected for all of these polypeptides, illustrating the broad interest in EGCG that led to a large body of experimental data that are available for mechanistic analysis.

7.2.2 Reduction in Cytotoxicity of Amyloidogenic Proteins

EGCG reduced the toxicity of amyloidogenic polypeptides related to a number of neurodegenerative diseases and systemic diseases that were listed above. EGCG treatment reduced cytotoxicity of Aβ (Levites et al. 2003; Ehrnhoefer et al. 2008; Bieschke et al. 2010) [see also Table 7.1], Htt (Ehrnhoefer et al. 2006), α-Syn (Ehrnhoefer et al. 2008; Bieschke et al. 2010; Lorenzen et al. 2014), IAPP (Meng et al. 2010) and tau (Wobst et al. 2015) in cellular models. Experiments probing the effect of EGCG on TTR toxicity in cell culture were inconclusive (Miyata et al. 2010).

Amyloid toxicity is believed to be linked to the presence of oligomeric aggregation intermediates (Walsh et al. 2002a; Haass and Selkoe 2007). However, the mechanism of toxicity has not yet been completely clarified. For Aβ and α-Syn, it was found that aggregates can permeabilize vesicular and mitochondrial membranes (Rodrigues et al. 2000; Volles et al. 2001). EGCG was shown to inhibit permeabilization of model membranes and mitochondrial membranes (Gauci et al. 2011; Caruana et al. 2011; Camilleri et al. 2013). In an *ex vivo* study with human semen samples from 47 individuals, the majority of samples contained SEVI species and EGCG was able to efficiently inhibit the SEVI-mediated HIV activity (Hauber et al. 2009).

7.2.3 Reduction of Amyloid Deposits and Toxicity in Higher Organisms

EGCG was shown to reduce amyloid deposition in animal models of protein misfolding disorders. Treatment with green tea extract rich in EGCG lowered the load of amyloid aggregates in *Caenorhabditis elegans* (Abbas and Wink 2010) and in AD mouse models (Rezai-Zadeh et al. 2005; Giunta et al. 2010; Dragicevic et al. 2011) [see also Table 7.1],

where it also reduced cognitive impairment (Rezai-Zadeh et al. 2008; Lee et al. 2009b). Depletion of amyloid deposits was observed in mouse models of Transthyretin Amyloidosis (Ferreira et al. 2012a, b), while depletion of amyloidogenic deposits in the human heart was observed in a phase II clinical trial after EGCG treatment (Kristen et al. 2012). Treatment with EGCG of a case of AL-Amyloidosis after several unsuccessful cycles of chemotherapy resulted in reduction of amyloid deposits in the patients' heart (Mereles et al. 2008). The same group performed a retrospective study on the influence of green tea and EGCG consumption on amyloid deposition in AL patients (Mereles et al. 2010). Phase II clinical studies by two European AL-Amyloidosis Treatment Centers are currently in progress (Schönland 2013; Merlini 2013). Phase II clinical studies have also been performed on AD, PD and HD (Chan 2007; Friedemann and Dörr 2009; Priller 2011).

7.2.4 Pharmacological Aspects of Aggregation Inhibition

Therapeutic use of EGCG is complicated by several pharmacokinetic drawbacks, most notably the widely variable bioavailability after oral consumption (Hunstein 2007). Nevertheless, it may be a treatment option worth evaluating, especially in rare diseases that are otherwise unprofitable for drug development. Exchanging the B-ring epigallo-moiety (Beecher 2003) by a gallo-moiety (Fig. 7.1) reduced the efficiency of the catechin on Htt aggregation (Ehrnhoefer et al. 2006), on Aβ aggregation (Bieschke et al. 2010) and Aβ cytotoxicity (Lin et al. 2009), and on SEVI-mediated infectivity (Hauber et al. 2009; Popovych et al. 2012). Depleting the C3-atom gallate function from the C-Ring showed comparable effects in the mentioned studies. In contrast to EGCG, Epigallocatechin (EGC) was neither able to disaggregate fibrils of the Sup35 NM25 strains prionogenic N-terminal domain (NM25) nor to inhibit its prion propagation (Roberts et al. 2009). EGCG treatment led to lysosomal degradation of PrP^{C} and subsequent accumulation of PrP^{Sc} in transiently transfected cell lines. While EGCG efficiently interfered with the PrP^{Sc} accumulation, four times the amount of EGC was necessary to achieve the same effect (Rambold et al. 2008).

7.3 Changes in Aggregate Morphology of Amyloid Precursors and Amyloidogenic Species

7.3.1 Folding State of Amyloid Precursors and Amyloidogenic Species

Protein misfolding can result in the formation of rope-like or straight amyloid fibrils that have highly stable cross-β sheet structures, which are aligned along the fibril axis (Chiti and Dobson 2006). The amyloidogenic species could either be a fragment of a precursor polypeptide like Aβ, Htt-exon1 and Insulin (Permutt et al. 1981), or entire proteins like α-Syn (Ulmer et al. 2005), LC protein (Redegeld and Nijkamp 2003) and TTR (Wojtczak et al. 1992). The native structure of amyloid precursors range from highly unstructured proteins like Htt (Zhang et al. 2013) and α-Syn (Dedmon et al. 2005) to highly structured β-sheet rich proteins like β2m (Bjorkman et al. 1987), TTR (Wojtczak et al. 1992) and LC (Edmundson et al. 1993), or α-helix rich proteins like the PrP (90–232) (Riek et al. 1996).

Irrespective of the starting point, the formation of the cross-β-sheet structure requires at least a partial unfolding or structural rearrangement of the amyloid precursor. The presence of EGCG during aggregation reduced the β-sheet content of all the protein aggregates for

which secondary structure was measured, namely α-Syn (Ehrnhoefer et al. 2008; Bieschke et al. 2010), MSP2 (Chandrashekaran et al. 2010, 2011), PrP (Rambold et al. 2008) and Insulin (Wang et al. 2012a).

7.3.2 Redirection of Aggregation into Non-amyloidogenic Species

The question of whether EGCG prevents the formation of amyloidogenic fibrils and aggregates was addressed in most studies with amyloidogenic polypeptides for which EGCG data was available. Its effect on *de novo* amyloid formation depends on the amyloidogenic protein (Fig. 7.2a). In most proteins, EGCG prevented amyloid fibril formation, and redirected the assembly process to generate amorphous aggregate species. This was observed for Htt (Ehrnhoefer et al. 2006), Aβ (Ehrnhoefer et al. 2008; Lopez del Amo et al. 2012), α-Syn (Bieschke et al. 2010; Suzuki et al. 2012), the HIV-mediating SEVI (Hauber et al. 2009; Popovych et al. 2012), IAPP (Meng et al. 2010; Suzuki et al. 2012), and Insulin (Wang et al. 2012a). EGCG was on the other hand unable to prevent fibril formation by β2m (Woods et al. 2011), the prion protein (Rambold et al. 2008), or heparin-induced fibril formation of tau (Wobst et al. 2015). However, EGCG completely prevented the formation of β-sheet rich aggregates of an aggregation-prone mutant tau (His-K18ΔK280) in the absence of heparin (Wobst et al. 2015). The crucial rate limiting step in TTR-amyloid formation involves monomerization of the TTR tetramer (Hammarstrom et al. 2003). Here too, EGCG redirected the aggregation of mutant TTR-L55P and TTR-Y78F from large amorphous aggregates into small amorphous aggregates (Ferreira et al. 2011). In another study, it prevented fibril formation of wt-TRR and TTR-V30M by preventing the monomerization of TTR (Miyata et al. 2010).

Remarkably the fibril formation of Sup35 NM25 was also redirected into amorphous aggregates, while fibril formation was not impaired by EGCG in the NM4 prion N-terminal domain (Roberts et al. 2009).

7.3.3 Remodeling of Pre-formed Amyloid Fibrils into Non-amyloidogenic Species

As discussed above, it is established that EGCG generally redirects the aggregation of an amyloid precursor. One could therefore ask whether it can also disrupt pre-formed amyloid fibrils, and whether such a disruption would result in similar supramolecular assemblies to those observed in aggregation in the presence of EGCG. Disaggregation of amyloid fibrils into comparable amorphous aggregate species was in fact reported in presence of EGCG for Aβ (Bieschke et al. 2010; Palhano et al. 2013), α-Syn (Bieschke et al. 2010; Caruana et al. 2011), tau His-K18ΔK280 (Wobst et al. 2015), IAPP (Meng et al. 2010; Cao and Raleigh 2012; Young et al. 2014), SEVI (Hauber et al. 2009) and the variants TTR-L55P and TTR-Y38F (Ferreira et al. 2011). Even fibrils of Sup35 were either disrupted and remodeled into large amorphous aggregates by EGCG (NM25), or not altered (NM4) corresponding to the results of *de novo* aggregation in presence of EGCG (Roberts et al. 2009). Additionally, EGCG was able to remodel preformed fibrils of MSP2 into large amorphous aggregates (Chandrashekaran et al. 2011).

7.3.4 Seeding Competence of EGCG-Induced Aggregates

A hallmark of amyloidogenic aggregates is their ability to incorporate monomeric polypeptides into amyloid structures. This process is referred to as ‘seeding-competence’, which is also a key property in prion propagation (Jarrett and Lansbury 1993). A simple test for seeding competence is to compare aggregation kinetics in the presence of a suspected amyloid precursor to those of unseeded aggregation (Ehrnhoefer et al. 2008). In mechanisms where nucleation is the rate-limiting step, the addition of seeding- or propagation-competent species will shorten or abrogate the lag phase.

Aggregates that resulted either from redirected monomer aggregation or from remodeling of mature fibrils by EGCG were either seeding-incompetent or showed reduced seeding-competence of Aβ (Ehrnhoefer et al. 2008; Bieschke et al. 2010; Palhano et al. 2013), α-Syn (Ehrnhoefer et al. 2008; Bieschke et al. 2010) and IAPP (Meng et al. 2010; Cao and Raleigh 2012). The prion protein (Rambold et al. 2008) as well as the yeast prion NM25 were also transformed into propagation-incompetent species, while the propagation-competence of NM4 was not impaired (Roberts et al. 2009).

7.3.5 Altered Aggregate Stability and Conformation

Resistance against denaturation by boiling in the presence of Sodium Dodecyl Sulfate (SDS-stability) is a rapid and useful indicator of aggregate stability (Wanker et al. 1999). Upon fibril formation, many amyloidogenic polypeptides become resistant to SDS denaturation and to proteolytic digestion by proteinase-K (PK-resistance). Changes in both properties would indicate structural changes in the aggregation process, although the structural basis of these effects is poorly understood. The SDS-stability can be measured either by a filter retardation assay (FRA) (Wanker et al. 1999) or SDS-PAGE (Ehrnhoefer et al 2008, Bieschke et al 2010). The latter is performed in the presence of SDS but without heating or at a temperature gradient, and can resolve differences in stability more sensitively. It is worth noting that apparent molecular weight in (semi-) denaturing gels does not necessarily reflect the size of the aggregates in solution.

The response of different amyloidogenic proteins to EGCG with respect to SDS and PK-stability varies considerably (Fig. 7.2b). EGCG prevented the formation of SDS-stable aggregates of Huntingtin (Ehrnhoefer et al. 2006) and an aggregation-prone tau mutant His-K18ΔK280, (Wobst et al. 2015). In contrast, EGCG induced highly SDS-stable aggregates of Aβ and α-Syn (Ehrnhoefer et al. 2008; Bieschke et al. 2010; Grelle et al. 2011; Gauci et al. 2011; Palhano et al. 2013). Similarly, we observed that EGCG accelerates the formation of SDS-stable species of immunoglobulin light chains (LC, Fig. 7.2b). SDS-stable aggregates of Aβ and α-Syn are structurally different from SDS-resistant fibrils these polypeptides form in the absence of EGCG. In LC aggregation, EGCG accelerates the formation of semi-SDS-stable aggregates. MSP2 was also reported to produce SDS-stable oligomer species in the presence of EGCG (Chandrashekaran et al. 2010, 2011). Rambold et al. (2008) found that EGCG reduced the PK-resistance of infectious PrP aggregates.

7.3.6 Conformational Change Can Be Probed by Antibody Binding

The anti-oligomer antibody A11 recognizes a common intermediate oligomeric structure in Aβ40 and Aβ42 (Kayed et al. 2003), Sup35 (Shorter and Lindquist 2004), β2m (Ribeiro et al. 2012), α-Syn (Ehrnhoefer et al. 2008) and tau (Flach et al. 2012; Wobst et al. 2015). EGCG prevented the binding of A11 to Aβ (Sinha et al. 2012), α-Syn (Ehrnhoefer et al. 2008) and tau (Wobst et al. 2015) indicating that either structural changes in the protein aggregates or EGCG binding obscured the antibody binding sites.

7.4 Towards a Holistic Molecular Mechanism for EGCG-Amyloid Interaction

7.4.1 Altered Protein Aggregation Kinetics by EGCG

The benzothiazole dye Thioflavin T (ThT) binds amyloid, amyloid fibrils and cross-β-sheet aggregation intermediates with a characteristic redshift of its emission spectrum, which does not occur in presence of unstructured monomers or oligomers (LeVine 1997). Therefore, it is frequently used to observe the aggregation kinetics leading to formation of amyloid fibrils by numerous amyloidogenic polypeptides (LeVine 1999).

EGCG reduced the ThT amplitude in most amyloidogenic polypeptides in a dose-dependent manner (see Table 7.1). This could be either due to a change in aggregate morphology, to partial sequestration of the amyloidogenic polypeptide or to an inhibition of ThT binding by competitive EGCG binding. After aggregation under influence of EGCG, the ThT fluorescence of Sup35 prion strains and TTR was reduced (Roberts et al. 2009; Miyata et al. 2010). By measuring kinetics of ThT fluorescence, it was shown that EGCG also slowed down the aggregation kinetics of Aβ (Ehrnhoefer et al. 2008; Grelle et al. 2011; Gauci et al. 2011; Sinha et al. 2012), α-Syn (Ehrnhoefer et al. 2008; Bieschke et al. 2010), IAPP (Meng et al. 2010; Suzuki et al. 2012; Young et al. 2014), tau (Wobst et al. 2015), MSP2 (Chandrashekaran et al. 2010, 2011), SEVI (Hauber et al. 2009) and β2m (Woods et al. 2011). These findings suggest that inhibition of aggregation kinetics might be due to a general mechanism of action of EGCG. EGCG however did not affect the kinetics of fibril formation of the tau protein when this was induced by heparin (Wobst et al. 2015). This is in contrast to the efficiency of EGCG in inhibiting the formation of ThT-positive tau oligomers in the absence of heparin, even at high substoichiometric concentrations (Fig. 7.2c) (Wobst et al. 2015).

When examining the effect of EGCG on different monoclonal immunoglobulin light chains isolated from urine of patients suffering either from AL Amyloidosis (clinical amyloid deposits) or from Multiple Myeloma (no clinical amyloid deposits), the formation of ThT-positive species was observed for all light chains (Andrich and Bieschke 2014). Typical aggregation kinetics showed a rapid phase of less than two hours followed by a slow phase of several days. In the presence of EGCG, only the ThT signal during the slow aggregation phase was reduced (Fig. 7.2c). The fact that light chains from patients without clinical amyloid deposits showed also the formation of ThT-positive species suggests that not every ThT-positive species of LC necessarily forms amyloid fibrils in patients.

We will discuss below how these different effects of EGCG on aggregation kinetics – inhibition, neutral, or acceleration – might be reconciled within a single model.

7.4.2 EGCG Interacts with Oligomeric Amyloid Precursors

EGCG binds to oligomeric species of Aβ, α-Syn and tau (Ehrnhoefer et al. 2008; Wobst et al. 2015). EGCG binding to oligomers was also reported for IAPP (Young et al. 2014), MSP2 (Chandrashekaran et al. 2010) and SEVI (Hauber et al. 2009).

A more difficult question to answer is, whether EGCG is able to bind to monomeric amyloid precursors. Nitro blue tetrazolium chloride (NBT) is a dye that can be used to stain EGCG (Paz et al. 1991). In aggregation assays, EGCG-specific staining by NBT was found for Aβ and α-Syn, and tau species that ran as monomers on SDS-PAGE (Ehrnhoefer et al. 2008). However, peptide species running as monomers on an SDS gel are not necessarily monomeric in solution. SDS-labile oligomers could bind EGCG and still disassemble during SDS-PAGE. These species would be distinct from monomers within the aggregation cascade. EGCG binding to native proteins, such as albumin has been observed at higher stoichiometric ratios (Ehrnhoefer et al. 2008; Bae et al. 2009; Nozaki et al. 2009).

EGCG binding to the aggregation-prone tau mutant His-K18ΔK280 resulted in monomers with a slightly lower electrophoretic mobility than the monomers prior aggregation, however the sub-stoichiometric effect of EGCG makes it likely that it preferentially binds to oligomeric aggregation intermediates rather than monomeric proteins (Wobst et al. 2015). Similarly, EGCG binding to the monomeric Aβ peptide was observed at a broad range of stoichiometries (Wang et al. 2010, 2012b), suggesting that multiple binding modes of EGCG exist to the Aβ peptide, possibly with a high affinity binding to oligomeric aggregation intermediates and lower affinity binding to the monomeric peptide. We postulate that the former high affinity interaction, rather than the latter low affinity interaction, is responsible for the inhibitory effect of EGCG on amyloid formation (Fig. 7.3a).

7.4.3 EGCG Remodels Aβ Fibrils Without Releasing Monomers or Oligomers

In the case of the Aβ peptides, it is well established that oligomeric intermediate species are cytotoxic (Walsh et al. 2002b; Haass and Selkoe 2007). We found that EGCG remodels fibrillar Aβ into spherical aggregates that are indistinguishable from those formed by monomeric Aβ in the presence of EGCG (Bieschke et al. 2010). Correspondingly, the remodeled aggregates exhibit reduced cytotoxicity. This would suggest that no toxic oligomeric species are produced during the remodeling of Aβ by EGCG. This hypothesis was tested by generating Aβ fibrils, each labeled by incorporating Aβ monomers that were linked either to green or red fluorophores. Mixtures of fibrils with both labels were incubated in presence of EGCG and the remodeling of fibrils was observed using fluorescence microscopy. If the remodeling process had involved the random dissociation of fibrils it would have resulted in oligomers in which red- and green-labeled monomers were statistically distributed. Instead, we observed that oligomers were clustered into either predominantly green or red, indicating that they had resulted from the remodeling of individual fibrils. Therefore, we concluded that EGCG directly remodels Aβ fibrils rather than disaggregating them into monomeric peptides (Bieschke et al. 2010). These findings are supported by Palhano et al. (2013), who were unable to detect by mass spectrometry Aβ40 monomers during remodeling.

7.4.4 Elucidating the Binding Mode of EGCG to Amyloid Fibrils and Precursors

7.4.4.1 Investigations of the Binding Mode of EGCG—A number of possible non-covalent and covalent binding modes for EGCG to misfolded polypeptides have been postulated: non-site specific interaction with exposed hydrophobic surfaces through the hydrophobic effect (Ehrnhoefer et al. 2008), site-specific hydrogen bonding (H-bonding) (Maiti et al. 2006), aromatic π-π-stacking (Scheraga et al. 1962), site-specific covalent binding, such as formation of disulfidebridges via cysteines (Lambert et al. 2008) and formation of Schiff bases via primary amines (Ishii et al. 2011). EGCG binding to amyloidogenic polypeptides has been analyzed using various biophysical and biochemical methods. For instance, isothermal titration calorimetry (ITC) was used to measure overall binding energies (Wang et al. 2010, 2012b). This approach however depends on highly homogenous preparations of interaction partners, which would be challenging when measuring interaction of EGCG with oligomeric amyloid precursors. For that reason, studies have so far been limited to monomeric proteins and peptides (Wang et al. 2010; Li et al. 2013). Peptide fragments have been engineered to address the question of site-specific binding (Wang et al. 2012b).

This approach must however, be interpreted with caution, since the results are only applicable to the native full-length polypeptide if the structure of the fragments resembles the structure of the parent polypeptide or if EGCG binds independently of the secondary structure formation. While monomeric Aβ, as many other amyloidogenic polypeptides, is unstructured as a monomer, this is not necessarily true for oligomeric aggregation intermediates.

Single amino acids have been substituted or chemically modified in Aβ and IAPP to analyze site-specific interaction of EGCG with the respective amino acids. Specifically, lysines were removed to probe for Schiff base formation (Wang et al. 2010, Wang et al. 2012b, Palhano et al. 2013), phenylalanines and tyrosines for π-π-stacking (Cao and Raleigh 2012, Lopez del Amo et al. 2012) and cysteins for SH-linkage (Cao and Raleigh 2012). Of course, this approach also relies on the assumption that the substitution does not alter the structure of the polypeptide within the amyloid oligomers and fibrils.

Solution-state and, more so, solid-state NMR spectroscopy provide technically challenging, but non-disruptive methods for probing the structure of EGCG-bound polypeptides (Lopez del Amo et al. 2012). Here, the solubility and size of the amyloid polypeptide as well as sample homogeneity and spectral resolution are limiting factors. Since all these methods have their constraints, it is necessary to combine the findings of different methods to draw a reliable picture.

7.4.4.2 EGCG is Binding to Cross-β-Sheet Motif in Aβ End-Stage Oligomers and Fibrils—Solid-state NMR (Lu et al. 2013; Petkova et al. 2006), X-ray crystallography (Colletier et al. 2011) as well as hydrogen-deuterium (H/D) exchange and mutational analysis (Luhrs et al. 2005) have elucidated Aβ fibrillar structure. Within the core of a fibril strand, the Aβ monomers form a hairpin structure (Fig. 7.1b). These hairpins are stacked on top of each other, in a way that peptide groups of adjacent monomers form intramolecular cross-β-sheet H-bonds while their amino acid side chains alternate towards the fibril surface

and towards the fibril center. The side chains between the arms of the hairpins interdigitate to further stabilize the fibril structure (Colletier et al. 2011).

The outwardly directed N-terminal β-sheet region of Aβ contains an LVFF-motif with Leu17 and Phe19 orientated towards the inward C-terminal β-sheet region, while Val18 and Phe20 point out towards the fibril surface. Lysine16 could in principle provide a Schiff-base anchor directly upstream to the LVFF-motif. The N-terminal amino acids 1–14 of Aβ are believed to be mostly unstructured and not part of the cross-β-sheet structure (Luhrs et al. 2005, Petkova et al. 2006). The N-terminal tail of Aβ contains a high degree of aromatic, acidic and basic amino acids, which could interact with EGCG through π-π-stacking or H-bonding. In contrast, hydrophobic side chains are concentrated on the C-terminus of the peptide that lies on the second arm of the hairpin (Petkova et al. 2006).

The structure of EGCG-induced Aβ oligomers was examined using solid-state NMR spectroscopy, which also yielded insights towards the binding model of EGCG (Lopez del Amo et al. 2012). As expected, no clear structure could be observed for the flexible N-terminal domain. In contrast, the LVFF motif was well-structured in the presence of EGCG. However, its structure was distinct from that in the absence of EGCG and the β-sheet structure of the KLVFFA region was lost in the presence of EGCG, while the β-sheet on the C-terminus of Aβ remained intact. Additionally the rotation of the phenyl rings (Phe19/20) as well as the rotation of adjacent His13/14 was hindered upon EGCG binding, strongly suggesting an interaction via π-π-stacking between the outwardly directed rings and the aromatic rings of EGCG. A cross-correlation for Phe19/Leu34 was found in presence and absence of EGCG, suggesting that the hydrophobic zipper stabilizing the hairpin structure may be partly intact in presence of EGCG.

Data stemming from ITC experiments conducted by Wang et al. (2010, 2012b) suggest that EGCG is forming H-bonds with the N-terminal domain of Aβ whilst binding to the LVFF motif via hydrophobic interactions. The authors proposed that the mode of EGCG binding gradually shifts from H-bonding to hydrophobic interaction at higher binding stoichiometries, which remains to be confirmed using complementary methods. The mean binding energies found in these ITC studies are lower than would be expected for the formation of covalent bonds, such as Schiff bases.

EGCG binding to Aβ40 via Schiff base formation was specifically investigated by acetylation of the two lysine residues Lys16 and Lys28 (Palhano et al. 2013) Lysine acetylation did not alter the effect of EGCG on ThT fluorescence, seeding competence or toxicity. Acetylation of Aβ results in blocking of Lys16 and Lys28 and prevents the salt-bridge formation of Aps23/Lys28 that is present in Aβ fibrils (Luhrs et al. 2005). Lys16 and Lys28 were mutated into arginine to prevent Schiff base formation, but and at the same time exclude an effect of the salt-bridge on EGCG activity. In the same study, Superoxide Dismutase (SOD1) was used to prevent EGCG oxidation. Neither modification prevented the remodeling of Aβ fibrils, yet both N-acetylation and Lys-to-Arg mutations prevented the formation of SDS-resistant aggregates. Moreover, treatment with SOD1 resulted in a delayed remodeling of pre-formed Aβ fibrils into SDS-stable aggregates (Palhano et al. 2013). It should be noted, however, that mutant SOD1 itself is capable of forming amyloid-

like fibrils associated with amyotrophic lateral sclerosis (ALS) (Falconi et al. 2013), so that SOD1 might compete with Aβ for EGCG binding. Taken together, these data suggest that covalent cross-linking by EGCG does occur, but is not required for remodeling of Aβ by EGCG.

7.4.4.3 Observations Towards the EGCG Mechanism in IAPP, α-Syn, MSP2 and SEVI—Fewer data are available for EGCG binding to other polypeptides. In IAPP, mutational analysis revealed that site-specific interactions (π-π-stacking, Schiff base formation, disulfide bridge formation) are not necessary for EGCG binding, which implies a non-covalent, non-site-specific binding mode via hydrophobic interactions (Cao and Raleigh 2012). Similarly, when the α-synuclein protein was incubated with EGCG about 30 % of all amino acids lost their NMR-resonances, suggesting an interaction that was not site-specific but likely driven by hydrophobicity (Ehrnhoefer et al. 2008). In an NMR-study with MSP2 only weak interaction of EGCG, mostly with the N-terminal and C-terminal region of the protein could be observed (Chandrashekaran et al. 2010). A pull-down experiment with EGCG-sepharose also found only weak interaction with MSP2; however, interaction might have been sterically hindered, since EGCG was coupled directly to the resin (Chandrashekaran et al. 2010). In contrast, Popovych et al. (2012) found that EGCG interacted with specific residues (amino acids 246–286) of the SEVI peptide, and that prevention of Schiff base formation in SEVI prevented the inhibition of fibrillation.

In conclusion, EGCG seems to interact with polypeptides both through hydrophobic modalities and by site-specific side-chain interactions, most likely through Schiff base formation. Whether covalent interaction or hydrophobic effect drives its anti-amyloid activity seems to depend on the specific polypeptide.

7.5 Conclusion – A Mechanism for Aggregation Redirection by EGCG

A large and growing body of scientific literature demonstrates that EGCG is a potent inhibitor of amyloid formation and amyloid toxicity. Early studies on Htt, α-synuclein and Aβ have identified a mechanism of EGCG that is distinct from other aggregation inhibitors, namely that EGCG redirects amyloidogenic polypeptides into highly stable off-pathway aggregates. Further studies have since characterized a variety of effects of EGCG on different polypeptides. This raises the question whether EGCG acts on amyloidogenic polypeptides by a plethora of mechanisms, or whether these effects may be combined into a unified picture.

Formation of amyloid fibrils proceeds by a common mechanism starting with an amyloidogenic monomer, that can be present under physiological conditions (α-Syn, PrP, Sup35), be released from a precursor as is the case for Aβ and TTR. Amyloidogenicity may result from elevated protein concentrations, as for immunoglobulin light chains and β-microglobin. Aggregation proceeds through a partially or fully unfolded monomeric state (*m*) to partially unstructured oligomer species (*o*) and then to cross-β-sheet oligomer species (*x*), which in the end progress into amyloid fibrils (*f*) (Fig. 7.3b). However, the relative stabilities of these aggregation intermediates result in aggregation kinetics with different rate-limiting steps for each polypeptide.

The variety in the details of the aggregation mechanisms may explain the apparent variety of mechanisms for the action of EGCG. The experimental data suggest that EGCG is indeed interacting with a common binding motif, but that the importance of the EGCG-binding species in the aggregation mechanism may be different depending on the polypeptide.

Data from several polypeptides indicate that EGCG binds to a cross-β-sheet structure, possibly at the same site and in competition with amyloidophilic dyes like ThT. Due to the sterical constraints in the cross-β-sheet structure, the hydrophobic side chains of the incorporated amino acids provide a distinct binding surface (Biancalana and Koide 2010). The fact that EGCG is able to remodel preformed fibrils of several amyloidogenic polypeptides implies its ability to bind the common cross-β-sheet structure. In our unified model, binding of EGCG to the cross-β-sheet oligomer (x) results in the species (x_E), which may then be remodeled into an amorphous aggregate (a_E) in case that this species is more stable than x_E (Fig. 7.3b).

Notably, the impact of the binding of EGCG to the amyloid cross-β-sheet motif depends on the energetic aggregation landscape of the specific amyloidogenic polypeptide (Fig. 7.3a). The shape of this landscape depends in turn on the relative stabilities of aggregation competent monomers, aggregation nuclei, oligomeric intermediates and fibrils.

Three modes of aggregation have been observed in studies involving EGCG: (1) the formation of the aggregation-competent species is rate-limiting (e.g. TTR, LC), (2) primary nucleation is rate limiting (e.g. tau), or (3) fibril propagation/secondary nucleation is rate limiting (e.g. Aβ, IAPP). The experimental data for these three mechanisms suggest different energetic states of the EGCG-induced aggregates (x_E) and different positions with respect to the rate-limiting step of aggregation (Fig. 7.3a).

If the formation of a cross-β-sheet nucleus is rate-limiting, then in binding to this rate-limiting species, the effect of EGCG on aggregation kinetics can be profound. The aggregation rate of tau, for instance, depends on the formation of an aggregation nucleus and it is likely that EGCG binds with strong affinity to the cross-β-sheet oligomers x. If EGCG binding remodels the nucleus into a non-amyloid aggregate, EGCG will inhibit aggregation at very low stoichiometric ratios, as is observed in the case of heparin-independent tau aggregation. In Fig. 7.2b, we see that EGCG prevents the large-scale formation of SDS-stable dimers (D), oligomers (O) and high-molecular-weight species (HMW) of tau, likely by sequestering a rate-limiting but non SDS-stable aggregation nucleus, leaving most of the protein in its monomeric state. We could not observe fibril formation under these conditions and therefore were unable to observe if fibril remodeling occurs. Therefore, we cannot be sure whether EGCG redirects tau nuclei into aggregates that have a lower energy than the fibrils.

In contrast, if the cross-β-sheet oligomer is formed after the rate-limiting step, the effect of EGCG on aggregation kinetics is much less potent. Whether EGCG has any effect on aggregation would depend on the relative stabilities of the EGCG-induced aggregate and the amyloid fibril. If the fibril is more stable, then EGCG can still bind to the cross-β-sheet motif, reducing ThT binding, but it will have no effect on aggregation kinetics; such is the

case for heparin-induced formation of tau fibrils (Wobst et al. 2015). The same may be true if formation of an aggregation competent species, for example a monomeric amyloid precursor, is rate-limiting. In this case, the rate-limiting species has no cross-β-structure and so EGCG binding does not affect aggregation kinetics. This is likely the case for TTR as well as for LC proteins.

In TTR, the rate-limiting step is the monomerization of the TTR tetramer (Hammarstrom et al. 2003). Under the experimental conditions of LC aggregation that we tested (neutral pH, mildly reducing conditions) the rate-limiting step in aggregation is the monomerization of LC dimers via reduction of disulfide bridges. Without a reduction of the disulfide bridges of light chain dimers, we did not observe aggregation over the course of one month, whereas the reduced protein formed ThT positive aggregates within hours. EGCG can then bind to this aggregate species and accelerate the formation of SDS-stable HMW-species (Fig. 7.2b).

In these cases, even if EGCG did bind to the amyloidogenic precursor, EGCG binding at substoichiometric concentrations cannot deplete its supply and is therefore not able to significantly slow down aggregation kinetics. Instead, the polyphenol seems to accelerate early aggregate formation but inhibit the slower aggregation into fibrillar species, which would be consistent with a nucleation-dependent mechanism for the second step of aggregation, although exact mechanistic details are yet to be explored.

Regarding the Aβ peptide, aggregation into non-β-sheet oligomers can occur rapidly, but nucleus formation is not rate limiting under most experimental conditions. Instead, secondary nucleation processes initiated by fibrillar species dominate the aggregation kinetics (Knowles et al. 2009; Cohen et al. 2013). Under these conditions, EGCG will inhibit aggregation kinetics if it can remodel fibrils into stable aggregates that are not seeding-competent, thereby removing the secondary nuclei. We found that fibril formation of Aβ is quantitatively redirected into SDS-stable amorphous aggregates, which are the most stable species on the aggregation pathway (Ehrnhoefer et al. 2008). Correspondingly, Aβ aggregation in presence of EGCG leads to formation of nonfibrillar aggregates (a_E, Fig. 7.2a). If these are more stable than the fibril, EGCG binding will also result in remodeling of EGCG bound fibrils (f_E) into amorphous aggregates (a_E). In the case of Aβ, EGCG bound to cross-β-sheet species partially disrupts the regular fibril structure and thus promotes the formation of aggregates with only partial β-sheet structure (Bieschke et al. 2010; Lopez del Amo et al. 2012). A similar mechanism may be applicable to IAPP, leading to the remodeling of fibrils into more stable EGCG-induced aggregates.

Our model would predict that the effect of EGCG on aggregation kinetics is lost if the fibrils are more stable than the EGCG-induced aggregates. This was indeed observed for heparin-induced tau fibril formation (Wobst et al. 2015) and may be the case for other amyloidogenic polypeptides for which EGCG would have no effect on aggregation kinetics, such as the NM4 strain of Sup35.

To conclude, the green tea polyphenol EGCG is an intriguing molecule that alters the amyloid aggregation process in novel ways. It has prompted a surprisingly large number of studies that scrutinize its activity on disease-related amyloidogenic proteins and peptides. Its

pleiotropic effects on the aggregation cascade of amyloidogenic polypeptides illustrates how subtle differences in the aggregation mechanism may yield very different outcomes of drug intervention. Thus, it remains to be explored whether the mechanistic insight provided by EGCG can be effectively translated into new therapeutic strategies.

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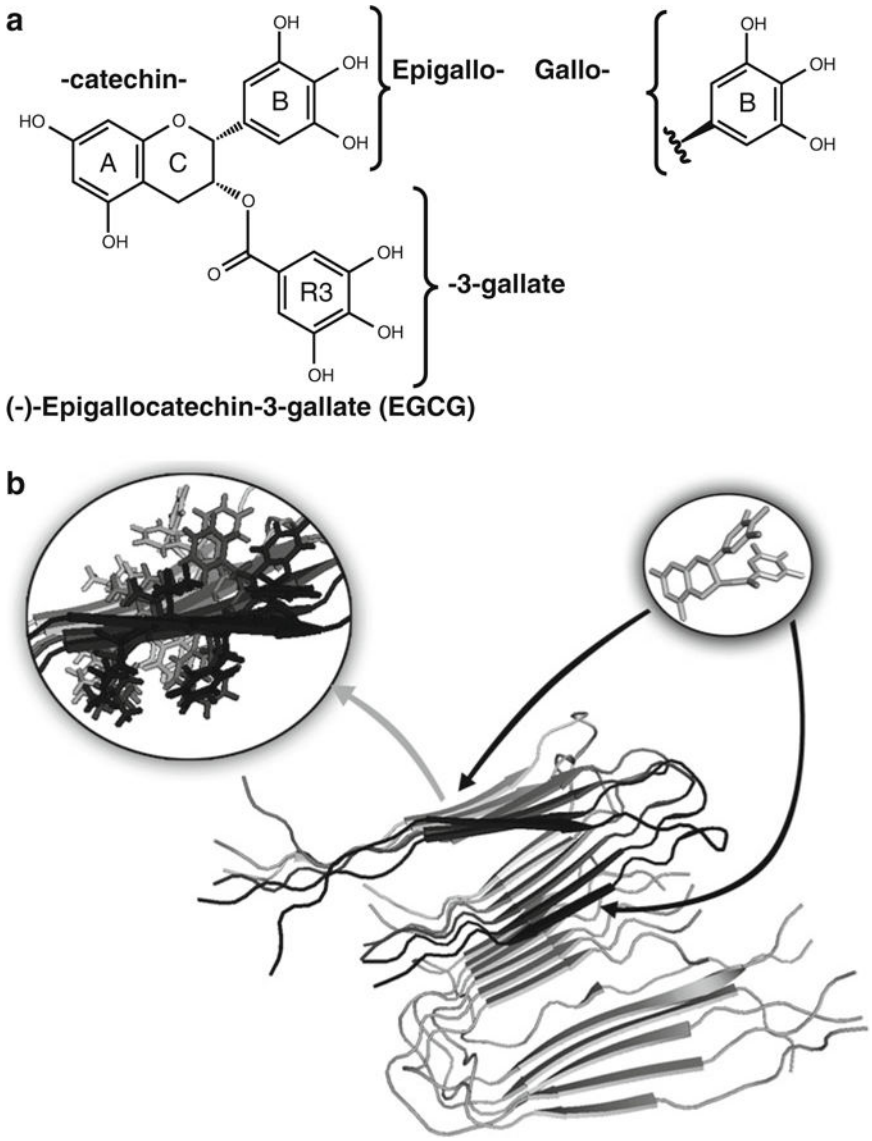

Fig. 7.1.

(−)-Epigallocatechin-3-gallate (EGCG) and its interaction with Aβ amyloid fibrils (**a**) EGCG is a flavonoid of the subclass flavan-3-ol having an unsaturated C-ring with the B-ring attached to its C2-atom. In EGCG, the B-ring is attached in epi conformation and the 3-ol of the C-ring is substituted by a 3-O-gallate function. The phenyl rings are di- or trisubstituted by phenol groups, thus making EGCG a polyphenol (Beecher 2003). (**b**) In Aβ fibrils, the monomers form a hairpin structure, with the N-terminal domain facing the fibril surface, while the C-terminal domain is directed towards the fibril core. Two or three monomers build the fibril base. The monomers within the fibrils are stacked on top of each other with adjacent monomers building intramolecular cross-β-sheets along the fibril axis (Lu et al. 2013; Petkova et al. 2006; Luhrs et al. 2005; Colletier et al. 2011). EGCG binding is indicated by black arrows. EGCG likely binds along the LVFF-motif (enhanced with side chain structure) (Grelle et al. 2011; Lopez del Amo et al. 2012; Wang et al. 2012b). This

figure was made using Pymol and structure coordinates were taken from PDB entry 2LMO (Petkova et al. 2006)

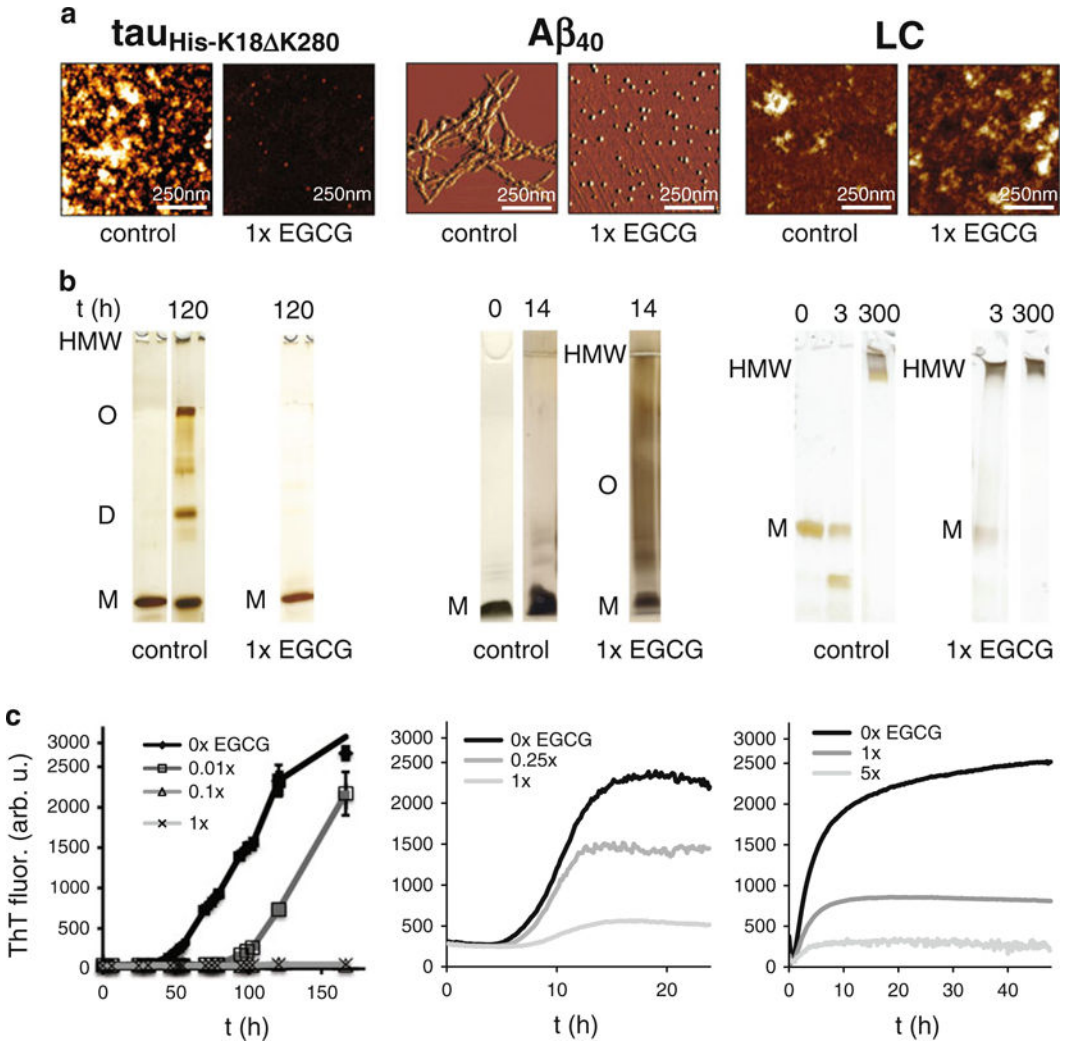

Fig. 7.2.

Influence of EGCG on aggregation kinetics, aggregate morphology and SDS-stability of tau, Aβ and LC. (**a**) Aggregation kinetics in absence (*black*) and presence (*gray*) of EGCG, where 1× mean equimolar concentrations of the amyloid precursor and EGCG. (**b**) Aggregate morphology after aggregation in absence and presence of EGCG. (**c**) SDS-stability (tau, Aβ) and semi-SDS-stability (LC, samples were neither reduced nor boiled) of aggregates formed in absence and presence of EGCG. With tau, EGCG prevents formation of SDS-stable aggregates while with Aβ it leads to SDS-stability indicating structural changes induced by EGCG. In LC, the formation of semi-SDS-stable aggregates was accelerated

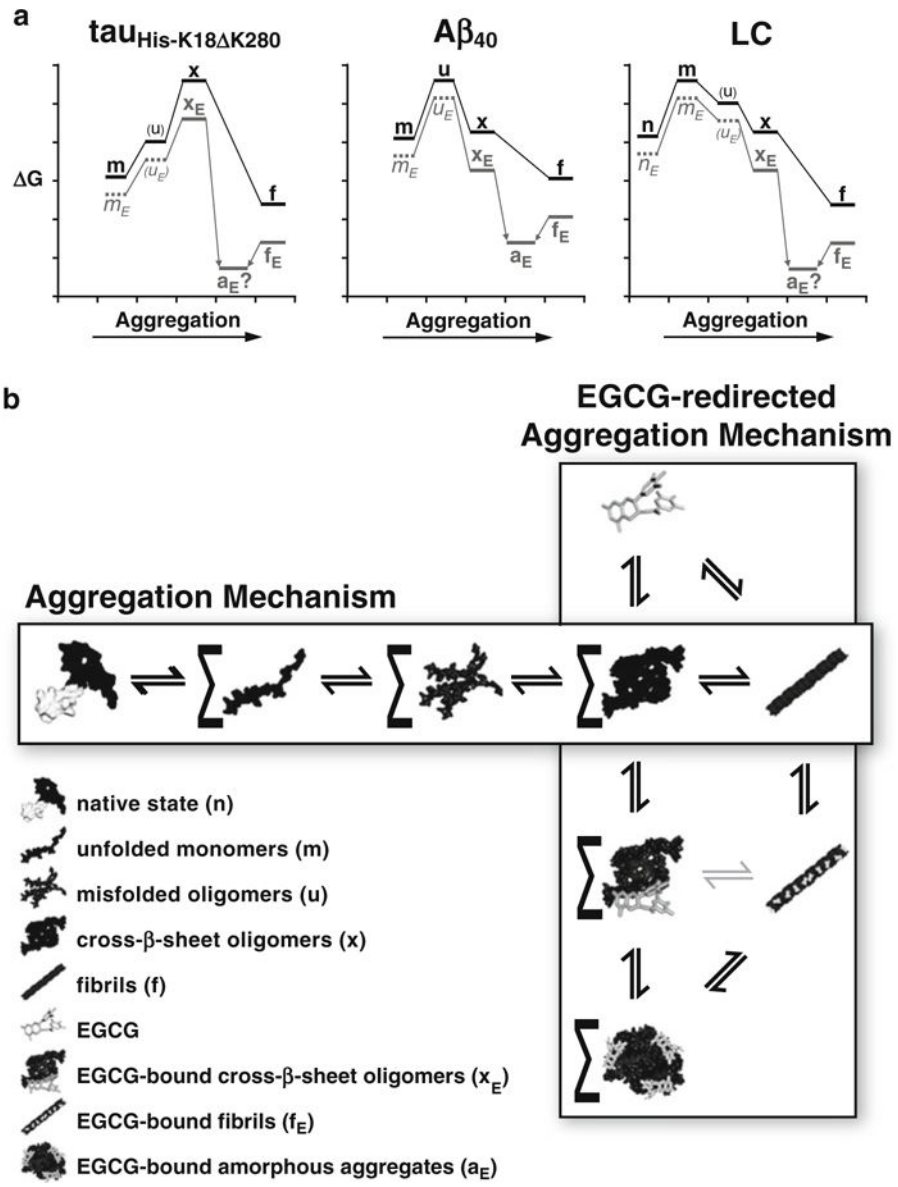

Fig. 7.3.

Redirection of amyloid formation by EGCG. (**a**) Simplified two-dimensional energy diagrams for the aggregation in absence (*black*) and presence (*gray*) of EGCG, with *n* indicating the native state, *m* partially unfolded monomers, *u* misfolded oligomers, *x* cross-β-sheet oligomers, *f* fibrils and the subscript *E* indicating an EGCG-bound state. Dashed energy levels imply that EGCG binds only with low affinity, for bracketed intermediates it is unclear whether they exist in a non-transient manner, question marks refer to species for which the stability in respect to the fibrillar state is not clear. (**b**) The formation of amyloid can be summarized in a common mechanism. Different intermediate subspecies are denoted by a summation sign. We propose that EGCG binds preferably to the cross-β-sheet oligomers and the cross-β-sheet motif on the fibril surface in the aggregation cascade.

Hence, EGCG disturbs the highly ordered fibril structure and induces the formation of EGCG-bound amorphous aggregates

Table 7.1

Effect of EGCG on disease-related polypeptides and prions (part 1)

Influence of EGCG	Htt	Aβ	α-Syn	IAPP
Aggregate morphology				
Prevents fibrils/aggregate formation		5	12, 23	25
Redirects into small amorphous aggregates		Y		
Leads to monomer depletion				
Disaggregates fibrils	1	12, 21	23	25, 26, 28
Remodels into large amorphous aggregates		12, 21	12	25, 26, 28
Kinetics				
Slows ThT kinetics		5, 14, 16, 17	5, 12	25, 27, 28
Accelerates ThT kinetics		N (5)	N (5, 12)	N (25, 27, 28)
Reduces or inhibits seeding-competence		5, 7, 18, 21	5, 12	25, 26
Inhibits prion propagation				
Structural				
Redirects into non-SDS-stable species	1	N (12)		
Redirects into semi-SDS-stable species		5, 16	5, 12	
Redirects into SDS-stable species		12, 14, 21	12, 14	
Reduces proteinase K-resistance				
Redirect into non-A11-specific oligomers		17	5	
Uncovers antibody binding epitope				
Reduces ThT/CR fluorescence amplitude		5, 12, 14, 16, 17, 21	5, 12	25, 27, 28
EGCG structure and binding				
Non-epi-gallo-moiety reduces efficiency	1	8, 12		
Gallate-moiety necessary for effectivity	1	8, 12		
Binds to oligomers		5	5	28
Direct remodeling		21, 12	12	
Interacts with specific residues		18		
Hydrophobic interactions		9, 18, 19		
H-Bonding		9, 19		
π-π stacking		18		N (26)
Schiff-base formation		N (9, 19, 21)		
Disulfide bridge				N (26)
Destruction of salt-bridges		N(9, 18, 19, 21)		
Toxicity				
Reduces membrane permeabilization		16, 20	20, 24	
Inhibits cytotoxicity (cell models)	1	10 papers[a]	5, 12, 24	25
Inhibits deposits in vivo (animal models)		3, 4, 6, 7, 10, 11, 15		
Reduces cognitive impairment in mice		4, 6		

Influence of EGCG	**Htt**	**Aβ**	**α-Syn**	**IAPP**
Inhibits deposits in vivo (humans)				
Phase 2 or 3 clinical trial	Y	Y	Y	

N() indicates studies with negative results, blank spaces indicate that no experimental results are available *Aβ* Amyloid-beta, *Htt* Huntingtin, *1APP* Islet Amyloid Polypeptide, *α-Syn* alpha-Synuclein; *1* (Ehrnhoefer et al. 2006), *2* (Levites et al. 2003), *3* (Rezai-Zadeh et al. 2005), *4* (Rezai-Zadeh et al. 2008), *5* (Ehrnhoefer et al. 2008), *6* (Lee et al. 2009b), *7* (Lee et al. 2009a), *8* (Lin et al. 2009), *9* (Wang et al. 2010), *10* (Giunta et al. 2010), *11* (Abbas and Wink 2010), *12* (Bieschke et al. 2010), *13* (He et al. 2011), *14* (Grelle et al. 2011), *15* (Dragicevic et al. 2011), *16* (Gauci et al. 2011), *17* (Sinha et al. 2012), *18* (Lopez del Amo et al. 2012), *19* (Wang et al. 2012b), *20* (Camilleri et al. 2013), *21* (Palhano et al. 2013), *22* (Lee et al. 2013), *23* (Caruana et al. 2011), *24* (Lorenzen et al. 2014), *25* (Meng et al. 2010), *26* (Cao and Raleigh 2012), *27* (Suzuki et al. 2012), *28* (Young et al. 2014)

[a] 2, 5, 8, 12, 13, 14, 15, 17, 18, 21

Table 7.2

Effect of EGCG on disease-related and polypeptides and prions (part 2)

Influence of EGCG	Sup35 NM25	Sup35 NM4	tau	MSP2	SEVI
Aggregate morphology					
Prevents fibrils/aggregate formation	30	N (30)	31		34, 36
Redirects into small amorphous aggregates			31		
Leads to monomer depletion					
Disaggregates fibrils	30	N (30)	31	33	34
Remodels into large amorphous aggregates	30	N (30)		33	34
Kinetics					
Slows ThT kinetics			31	32, 33	34
Accelerates ThT kinetics			N (31)	N (32, 33)	N (34)
Reduces or inhibits seeding-competence	30	N (30)			
Inhibits prion propagation	30	N (30)			
Structural					
Redirects into non-SDS-stable species					
Redirects into semi-SDS-stable species					
Redirects into SDS-stable species				32, 33	
Reduces proteinase K-resistance					
Redirect into non-A11-specific oligomers			31		
Uncovers antibody binding epitope			31		
Reduces ThT/CR fluorescence amplitude	30	30	31	32, 33	34
EGCG structure and binding					
Non-epi-gallo-moiety reduces efficiency					34, 36
Gallate-moiety necessary for effectivity	30	30			34, 36
Binds to oligomers			31	32	34
Direct remodeling					
Interacts with specific residues				32	34
Hydrophobic interactions				32	
H-bonding					

Influence of EGCG	Sup35 NM25	Sup35 NM4	tau	MSP2	SEVI
π-π stacking					
Schiff-base formation				N (32)	−34
Disulfide bridge				N (32)	
Destruction of salt-bridges					
Toxicity					
Reduces membrane permeabilization					
Inhibits cytotoxicity (cell models)			31		
Inhibits deposits in vivo (animal models)					
Reduces cognitive impairment in mice					
Inhibits deposits in vivo (humans)					
Phase 2 or 3 clinical trial					

N() indicates studies with negative results; blank spaces indicate that no experimental results are available *Sup35 NM25* prion strain (head region 21–38, center region 39–90, tail region 91–106), *Sup35 NM4* prion strain (head region 21–38, center region 39–78, tail region 79–96), *tau* HisK18ΔK280 fragment of tau protein, *MSP2* Plasmodium falciparum merozoite surface protein 2, *SEVI* semen-derived enhancer of virus infection, *30* (Roberts et al. 2009), *31* (Wobst et al. 2015), *32* (Chandrashekaran et al. 2010), *33* (Chandrashekaran et al. 2011), *34* (Hauber et al. 2009), *36* (Popovych et al. 2012)

Table 7.3

Effect of EGCG on disease-related polypeptides and prions (part 3)

Influence of EGCG	LC	TTR	β2-m	PrP	Ins
Aggregate morphology					
Prevents fibrils/aggregate formation		43	N (49)		51
Redirects into small amorphous aggregates		44			51
Leads to monomer depletion		43			N (51)
Disaggregates fibrils					
Remodels into large amorphous aggregates		44			
Kinetics					
Slows ThT kinetics	N (41)		49		
Accelerates ThT kinetics	41		N (49)		
Reduces or inhibits seeding-competence				50	
Inhibits prion propagation					
Structural					
Redirects into non-SDS-stable species	N (41)				
Redirects into semi-SDS-stable species	41	43			
Redirects into SDS-stable species	N (41)				
Reduces proteinase K-resistance				50	
Redirect into non-A11-specific oligomers					
Uncovers antibody binding epitope					
Reduces ThT/CR fluorescence amplitude	41	43	49		
EGCG structure and binding					
Non-epi-gallo-moiety reduces efficiency					
Gallate-moiety necessary for effectivity				50	
Binds to oligomers		43			
Direct remodeling					
Interacts with specific residues					
Hydrophobic interactions					
H-bonding					

Influence of EGCG	**LC**	**TTR**	**β2-m**	**PrP**	**Ins**
π-π stacking					
Schiff-base formation					
Disulfide bridge					
Destruction of salt-bridges					
Toxicity					
Reduces membrane permeabilization					
Inhibits cytotoxicity (cell models)					
Inhibits deposits in vivo (animal models)		45, 47			
Reduces cognitive impairment in mice					
Inhibits deposits in vivo (humans)	37, 38	46			
Phase 2 or 3 clinical trial	Y	Y			

N() indicates studies with negative results; blank spaces indicate that no experimental results are available *LC* immunoglobulin Light chain protein, *TTR* transthyretin (studies with variants: wt/V30M/E54K/Y38F/L55P), *β2-m* β2-Microglobulin, *PrP* prion protein, *Ins* Insulin *37* (Mereles et al. 2008), *38* (Mereles et al. 2010), *41* (unpublished data), *43* (Miyata et al. 2010), *44* (Ferreira et al. 2011), *45* (Ferreira et al. 2012b), *46* (Kristen et al. 2012), *47* (Ferreira et al. 2012a), *49* (Woods et al. 2011), *50* (Rambold et al. 2008), *51* (Wang et al. 2012a)