Protein interactions in the assembly of the tail of bacteriophage T4

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Abstract Protein interactions in the assembly of the baseplate have been investigated. The baseplate of the phage T4 tail consists of a hub and six wedges which surround the former. Both reversible and irreversible interactions were found. Reversible association includes gp5 and gp27 (gp: gene product) which form a complex in a pH-dependent manner and gp18 polymerization, i.e. the tail sheath formation depends on the ionic strength. These reversible interactions were followed by irreversible or tight binding which pulls the whole association reaction to complete the assembly. The wedge assembly is strictly ordered which means that if one of the seven wedge proteins is missing, the assembly proceeds to that point and the remaining molecules stay non-associated. The strictly sequential assembly pathway is suggested to be materialized by successive conformational change upon binding, which can be shown by proteolytic probe.

Keywords Protein interaction · Protein assembly · Reversible association · Bacteriophage · Contractile tail · Analytical ultracentrifugation

Introduction

Assembly of protein oligomers is in general a spontaneous process which does not require energy. Assembly or association requires a loss of entropy of the associating protein molecules.

If the association is favored, i.e. a favorable free energy change, then the reaction must include either a negative enthalpy change and/or a positive entropy change due to the structural changes of water to compensate the inherent loss of system entropy arising from protein assembly. The favorable negative enthalpy change generally comes from weak non-covalent interactions, such as electrostatic interactions, hydrogen bonds and van der Waals interactions or dispersion forces, and favorable positive entropy change comes from hydrophobic interactions at the contact regions of the associating proteins.

The interaction for oligomerization is highly specific in spite of the extremely crowded environment in the cell. In fact, specificity is the most important characteristic for biological interactions. There are a sizable number of protein supramolecules in the cell, including the ribosome, proteasome, replisome, cyclosome, etc., as well as bacterial flagella and viruses. Subunits of these supramolecules function cooperatively to materialize special functions which are not feasible with each constituent subunit by itself. The first supramolecule which was intensively studied with regard to its assembly was TMV by Fraenkel-Conrat and Williams 1955. They separated TMV capsid protein subunits and RNA and found that a viable virus was self-assembled when the monomeric protein subunits and the detergent-extracted RNA were mixed. This discovery led to the concept of “self-assembly”, and since then it has been widely recognized that the biological structures in the cell are, in general, self-assembled.

In 1964, conditional lethal mutants of phage T4, temperature sensitive and amber, were isolated and these were used to study the assembly (Epstein et al. 1964). This situation allowed the phage to be grown under permissive conditions to enable the function of the gene product to be subsequently determined upon switching to the non-permissive conditions (where the gene product is missing). Upon T4 infection, protein synthesis of the bacterium is immediately stopped and the phage proteins start to be synthesized. Phage proteins can thus be specifically labeled by ^{32}S - or ^{14}C -labeled amino acids added after

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infection. The labeled lysates were subjected to sucrose-density gradient centrifugation (SDG) and the proteins were identified by SDS-PAGE. Based on this combination of SDG and SDS-PAGE, Kikuchi and King (1975a, b, c) elucidated the assembly pathway of the tail of phage T4. In vivo assembly of the tail was later studied by Ferguson and Coombs (2000), who utilized the pulse-chase method and confirmed the in vitro assembly of the tail. The assembly pathway of the wedge is strictly sequential which means that, if one of the constituent subunit proteins is missing, the intermediate structure up to that point is formed, but all other proteins to be added in the later stage will remain unassociated. Head assembly takes place independently on the inner membrane of the host *E. coli*, and some of the head proteins, including the major capsid proteins, gp23 and gp24, go through processing. Processing involves the cleavage, by gp21 the T4 prohead protease, of their amino terminal residues, approximately 60 and 20 residues from gp23 and gp24 respectively (Showe et al. 1976). There is no such processing in tail proteins except for gp5, where the peptide bond between residue S351 and A352 is cleaved. In this case, the C-terminal triple-stranded β -helix domain is not lost upon cleavage, but is instead retained and plays an important role in creating a hole by functioning as a needle upon infection (Kanamaru et al. 1999). The completed tail binds to the portal vertex of the head and the resultant tail fiberless phage will bind six tail fibers together with whiskers to form a viable phage. In T4 phage assembly, the only known step of assembly which requires energy is the DNA packaging, where ATP splitting into ADP and Pi provides the free energy, which is utilized by the DNA packaging motor, gp17/gp16 complex, to package DNA into the head (Rao and Black 1988). The assembly mechanism of bacteriophage is thus much more complicated than simple RNA viruses and the assembly is intricately regulated.

The structure of the tail and many of the constituent subunit proteins have been determined in Michael G. Rossmann's laboratory at Purdue University in collaboration with Vadim V. Mesyanzhinov's and our groups, as well as in Mark van Raaij's laboratory (Kostyuchenko et al. 2003, 2005; Rossmann et al. 2007; Leiman et al. 2010). X-ray crystal structures of 14 out of more than 40 structural proteins have been completely or partially determined and located in the lower resolution electron density of the whole phage particle as determined by 3D reconstruction from electron micrographs.

Strictly sequential assembly of the baseplate wedge of the tail of phage T4

There are seven gene products, gp11, gp10, gp7, gp8, gp6, gp53, and gp25, which assemble to form a wedge in this order except that gp11 can bind to gp10 regardless of the assembly state of gp10 (Kikuchi and King 1975a; Table 1). Gp11 and

gp10 are trimeric, gp8 and gp6 are dimeric, and gp7, gp53, and gp25 are monomeric in solution (Zhao et al. 2000). "Strictly sequential" implies that if, e.g. gp6, is missing in this system, $(gp11)_3(gp10)_3(gp7)(gp8)_2$ is formed and gp25 and gp53 remain unassociated. In order to reexamine the sequential assembly of the wedge, all the seven genes which encode the corresponding gene products were cloned into expression vectors and expressed. For gp53 and gp25, the yield of the expressed proteins was not high enough to perform physical measurements and also tended towards non-specific aggregation. Yap et al. (2010a, b) have thus devised a new method to examine the interactions and to obtain the complex. Genes were expressed separately and the cells which express different genes were first mixed and then lysed. For details of the experiments, see Yap et al. (2010a). The results are shown in Table 2. We call interactions which are expected from the strictly sequential assembly as "legitimate interactions" as opposed to "non-legitimate interactions". It is noted that no "non-legitimate" interactions except for those among gp6, gp53, and gp25 were observed, which will be discussed in the next section in terms of the basis for strictly sequential pathway. All the intermediate complexes in the wedge formation were stable and no dissociation was observed by analytical ultracentrifugation (Yap et al. 2010b). On the other hand, interaction between gp5 and gp27, which are the major components of the hub, was not very strong as observed by sedimentation equilibrium (Fig. 1).

Experimental evidence for the conformational change as the basis of the strictly ordered pathway

Table 1 indicated that gp6, gp53, and gp25 interact with each other, but the interactions are not so strong as to isolate the ternary complex of the three gene products. Gp6, however, binds gp53 much more strongly than gp25 when gp6 is bound to the precursor complex, i.e. $(gp10)_3(gp11)_3(gp7)_1(gp8)_2$. This supports the idea that the legitimate assembly pathway is materialized mainly by conformational change upon association. The conformational change upon association is more directly observable by examination of the protease susceptibility. For example, gp10 and gp7 are both lysylendopeptidase-sensitive. However, when gp10 binds to gp7, it becomes totally resistant to protease (Kanamaru et al., in preparation; Yap et al. 2010a, b). On the other hand, gp7 is still susceptible to lysylendopeptidase in the $(gp10)_3(gp7)_1$ complex and interestingly, a small region of gp7 at the C-terminal region with the molecular weight of 14,000 remains bound to gp10 which made gp10 totally resistant to lysylendopeptidase. This is most easily understood based on the conformational change of gp10 upon binding the small domain of gp7. Each step in the assembly of the wedge is irreversible.

Table 1 Tail proteins

Gene product (gp)	a.a. residue number	Stoichiometry in the tail	Localization or function	PDB
gp11	219	18 (3) ^a	Wedge	1EL6
gp10	602	18 (3)	Wedge	2FKK
gp7	1032	6 (1)	Wedge	
gp8	334	12 (2)	Wedge	1N7Z
gp6	660	12 (2)	Wedge	3H2T
gp53	196	6 (1)	Wedge	
gp25	132	6 (1)	Wedge	
gp5	575	3	Hub (tail lysozyme)	1K28
gp27	391	3	Hub	1K28
gp29	590	3	Hub (tape measure prtotein)	
gp26	208	ND	Hub (?)	
gp28	177	ND	Hub (?)	
gp9	288	18	Plug for tailfibers	1S2E
gp12	527	18	Short tailfiber	1H6W, 1OCY
gp48	364	6	Baseplate	
gp54	320	6	Baseplate (tube initiator)	
gp18	659	138	Sheath protein	3FOA
gp19	163	138	Tube protein	
gp3	176	6	Tube terminator	
gp15	272	6	Sheath terminator	
gp57A	79	-	Chaperone	
gp51	249	ND	Catalytic	

ND not determined

^aThe numbers in parentheses are per wedge

Formation of the baseplate-like structure from wedge intermediates

As the strictly sequential assembly of the wedge proceeds, the sedimentation coefficients of the intermediate complex increased as expected (Table 2), but it was found that the association of gp53 to the immediate precursor complex formed an expected 15.0S complex, but also induced spontaneous association of the six wedges to form a six-fold star-shaped baseplate-like structure with the s-value of 43.7S in the absence of the hub (Yap et al. 2010, Table 3). The formation of the baseplate-like structure was facilitated by the addition of

gp25. The wedge in the supramolecular complex has a structure similar to the baseplate in the extended “star” conformation after infection. The biological significance of the baseplate-like structure is not clear, but the wedge has the ability to form a baseplate.

pH-dependent reversible association of gp5 and gp27

Gp5, the tail lysozyme (Nakagawa et al. 1985), and gp27 form a hetero-hexameric complex, (gp5)₃(gp27)₃ (Kanamaru et al. 1999, 2002), which constitutes the major part of the hub. Gp5 subunit consists of three domains, the

Table 2 Interactions among wedge proteins

Protein/protein complex	gp11	gp7	gp8	gp6	gp53	gp25
gp10	++	++	–	–	–	–
gp7	–		–	–	–	–
gp8	–			–	–	–
gp6	–				+	+
gp53	–					+
gp10–gp7	++		++	–	–	–
gp10–gp7–gp8	++			++	–	–
gp10–gp7–gp8–gp6	++				++	–
gp10–gp7–gp8–gp6–gp53	++					++

++ strong interaction, + weak interaction, – no interaction

Table 3 Sedimentation coefficients of wedge intermediate complexes

Complex	S _{20w}
gp10–gp7	10.2 S
gp10–gp7–gp8	12.1 S
gp10–gp7–gp8–gp6	14.5 S
gp10–gp7–gp8–gp6–gp53	15.0 S, 43.7 S
gp10–gp7–gp8–gp6–gp53–gp25	15.3 S, 42.0 S
gp11–gp10	9.7 S
gp11–gp10–gp7	11.9 S
gp11–gp10–gp7–gp8	13.4 S
gp11–gp10–gp7–gp8–gp6	15.3 S
gp11–gp10–gp7–gp8–gp6–gp53–gp25	16.0 S, 40.6 S

N-terminal, the lysozyme, and the C-terminal domain. During assembly, gp5 undergoes processing, where the linker between the lysozyme- and the C-terminal domains is nicked between Ser351 and Ala352, but the resultant two polypeptides stay together until infection (Kanamaru et al. 1999, 2005). The C-terminal domain which forms a triple-stranded β -helix plays the role of a needle to puncture a hole in the outer membrane upon infection and locally digging a hole in the peptidoglycan layer, which makes it possible for the tail tube to reach the inner membrane. When the heterononameric complex (gp27)₃(gp5*)₃(gp5C)₃ (the asterisk denotes the processed gp5) is incubated at 37 °C for half an hour (See Sarkar et al. 2006), it dissociates into three (gp27)(gp5*) hetero-dimers and (gp5C)₃. Gp5* contains the N-terminal and a lysozyme domain. The interaction between gp27 and gp5* is pH-dependent and the hetero-dimer complex (gp27)(gp5*) is stable at pH 8.6 (See Sarkar et al. 2006), but it dissociates into each monomer, gp27 and gp5* at pH 6, which would be physiologically relevant, because the lysozyme moieties should be free in the periplasm (where the pH is low) and would digest the peptidoglycan layer, so that the tail tube can penetrate to reach the inner membrane.

Fig. 1 Sedimentation equilibrium of gp27–gp5* complex at pH 6 and pH 8

Ionic strength-dependent association of gp18, the tail sheath protein, onto the tube–baseplate

When tails, isolated from the head-deficient mutant phage-infected *E. coli*, are dialyzed against low-ionic strength buffer, the tail sheath dissociates from the tube–baseplate into monomeric gp18 (Tschopp et al. 1979). This dissociation is reversible, and when the tube–baseplate and the monomeric gp18 are mixed and the KCl concentration is increased to 0.1 M, the tail sheath is reassembled (Arisaka et al. 1979). The association and dissociation is highly cooperative in that, when the association reaction is observed by electron microscopy, either complete tails or naked tube–baseplates are observed, but the sheath with intermediate length were extremely rare. The cooperative association was confirmed by sedimentation velocity, where only 120S tails and 72S tube–baseplate were observed and no molecular species with intermediates between the two were seen. Whereas the tail sheath is readily dissociable from the isolated tail, the tail sheath in the virion does not dissociate. It is surmised that there must be another step, perhaps the head joining, which makes the extended tail sheath highly stable against dissociation. The crystal structure of gp18 was later solved by Aksyuk et al. (2009).

Tube polymerization terminator, gp3, and connector protein, gp15

The tube protein, gp19, polymerizes onto the baseplate. Although the detailed mechanism is not known, it is generally believed that, as the tail tube polymerizes, the tape measure protein, gp29 (Abuladze et al. 1994) (which originally has a compact shape), elongates itself and, when it is completely extended, gp3 binds to the tip of gp29 and gp19. Concurrently, gp18 polymerizes onto the tube–baseplate complex and when the sheath formation completes, gp15, the connector protein binds to the tip of gp19 and gp18. When expressed in the cell, both gp3 and gp15 spontaneously form hexamer rings (Zhao

et al. 2003) which are highly stable. The X-ray structure of gp15 has very recently been solved (Fokine et al. 2013).

Reversible association versus irreversible association in the tail assembly

Both reversible and irreversible associations were observed along the pathway of the tail assembly. Two examples for reversible association described above were gp27–gp5 interaction and the tail sheath protein gp18 polymerization onto the tube–baseplate. In the former case, it is very likely that gp5* will dissociate upon infection from gp27 and will be released into the periplasm to locally dig a hole in the peptidoglycan layer. In the case of the tail sheath formation or gp18 polymerization, the final product, the extended tail sheath, is a metastable structure and undergoes contraction upon infection, where sixfold symmetrically arranged gp18 subunits slide each other to transform into the extremely stable contracted form which has a pseudo-12fold symmetry. The large free energy difference of the two conformations would make the contraction spontaneous upon its triggering by the conformational change of the baseplate. In either case, the relatively weak interactions in the stable virion make the functional structure change possible. The reversible interactions must be followed by tight interactions that pull the assembly pathway towards completion, and the final product, the virion, is an extremely stable structure. Reversible interactions or relatively weak interactions appear to be utilized, where the interaction retains the ability to undergo structural change,

Conclusion

The tail of bacteriophage T4 has been investigated as a model system for a protein supramolecule. One of the major functions of the virion is to protect its genomic DNA from harsh environmental perturbations including DNase attack after release from the host cell. Virions or capsids are, therefore, in general quite stable both physically and chemically. Another function which is especially endowed to the tail is to efficiently transfer its DNA into the host cell. As we saw, both reversible and irreversible interactions are observed in the assembly of the tail and it appears that the reversible or weak interactions are utilized where conformational change or dissociation are functionally required for the tail upon infection, whereas the irreversible or tight interactions are used for assembly of parts to form sub-assembly, e.g. wedge, which have a rigid structure and behave as a unit. The stoichiometry, the binding strength and molecular size and shape properties are conveniently investigated by analytical ultracentrifugation.

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Conflict of interest There is no conflict of interest on this paper.

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