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Purification of recombinant G-protein-coupled receptors

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Abstract

Structural and functional analysis of most G-protein-coupled receptors (GPCRs) requires their expression and purification in functional form. The produced amount of recombinant membrane-inserted receptors depends on the optimal combination of a particular GPCR and production host; optimization of expression is still a matter of trial-and-error. Prior to purification, receptors must be extracted from the membranes by use of detergent(s). The choice of an appropriate detergent for solubilization and purification is crucial to maintain receptors in their functional state. The initial enrichment can be carried out by affinity chromatography using a general affinity tag (e.g. poly-histidine tag). If the first purification step does not yield pure receptor protein, purification to homogeneity can often be achieved by use of a subsequent receptor-specific ligand column. If suitable immobilized ligands are not available, size exclusion chromatography or other techniques need to be applied. Many GPCRs become unstable upon detergent extraction from lipid membranes, and measures for stabilization are discussed. As an example, the purification of a functional neurotensin receptor to homogeneity in milligram quantities is given below.

Introduction

Structure determination and functional analysis of integral membrane proteins, which are not naturally abundant, require (i) a recombinant production system and (ii) a purification strategy to allow the isolation of functional rather than non-functional, incorrectly folded membrane protein. Expression and purification of prokaryotic and eukaryotic membrane proteins has been covered in the literature. The reader is referred for example to (Grisshammer and Tate, 1995; BBA Special Issue on Overexpression of Integral Membrane Proteins, Tate and Grisshammer (eds.), 2003; Structural Biology of Membrane Proteins, Grisshammer and Buchanan (eds.), 2006). In addition, the reader may consult Chapter [33] by Jack Greenblatt (Protein-protein interaction chromatography) and Chapter [41] by Sue-Hwa Lin (Purification of membrane proteins and purification of glycoproteins) in this volume. This chapter focuses exclusively on G-protein-coupled receptors (GPCRs) which are eukaryotic integral membrane proteins involved in cell-to-cell communication and sensory signal transduction (see reference (Gether and Kobilka, 1998)).

It is beyond the scope of this chapter to discuss in any great detail all the possible expression strategies for integral membrane proteins such as GPCRs. However, a few key points are summarized below. (i) A universal strategy for the high-level recombinant expression of functional receptors is currently unavailable. Some GPCRs accumulate in the membrane to

high levels, whereas other often closely related receptors are hardly detected. Despite their assumed similarities, individual GPCRs behave quite differently in a given expression host and recombinant production is still a matter of trial-and-error. Comparative expression studies have for example been performed using the methylotrophic yeast *Pichia pastoris* (Andre *et al.*, 2006), the baculovirus insect cell system (Akermoun *et al.*, 2005), and the Semliki Forest virus system (Hassaine *et al.*, 2006). A survey of GPCR production in commonly used expression hosts has been summarized (Sarramegna *et al.*, 2003). (ii) The use of eukaryotic hosts seem generally better for producing functional, membrane-inserted GPCRs than prokaryotic hosts (Grisshammer, 2006), although, there are exceptions. For example, the bacterium *Escherichia coli* (*E. coli*) was successfully used for the expression of the neurotensin receptor NTS1 (Grisshammer *et al.*, 1993; White *et al.*, 2004), the M1 muscarinic acetylcholine receptor (Hulme and Curtis, 1998), the adenosine A2a receptor (Weiß and Grisshammer, 2002), and the cannabinoid CB2 receptor (Calandra *et al.*, 1997; Yeliseev *et al.*, 2005). (iii) Numerous descriptive reports have been published on the recombinant expression of integral membrane proteins. However, few publications (see references (Griffith *et al.*, 2003; Bonander *et al.*, 2005; Wagner *et al.*, 2006; Wagner *et al.*, 2007; Bonander *et al.*, 2009)) are currently available to understand the underlying mechanisms of how a given host cell responds to membrane protein overproduction.

The purification of receptors can be conceptually divided into two steps: Extraction from membranes with a suitable detergent (solubilization), and subsequent purification by use of general affinity tags, receptor-specific ligand columns, size exclusion chromatography and other methods. Of utmost importance, care must be taken to choose experimental conditions to maintain the membrane protein in its active state throughout the purification procedure. This latter aspect cannot be over-emphasized because many GPCRs become unstable upon detergent extraction from lipid membranes.

Solubilization – General Considerations

The extraction of membrane-inserted receptors is accomplished with the use of detergents (see Chapter [40] by Dirk Linke (Detergents: An overview)). The correct choice of detergent is crucial to maintain solubilized receptors in their functional state for a prolonged period of time. N-dodecyl- β -D-maltoside (DDM), a mild non-ionic detergent, is commonly used for the solubilization of GPCRs. Lipid-like cholesteryl-hemisuccinate may be added to increase receptor stability (see below). The use of shorter-chain detergents, which are usually harsher than longer-chain detergents, is appropriate as long as these detergents do not compromise the integrity of the GPCR under investigation.

Solubilized receptors must be considered as detergent-lipid-receptor complexes rather than just as receptor protein. This implies that the biochemical properties such as size, shape, or isoelectric point of solubilized receptors differ from those computed solely by the amino acid sequence. Likewise, detergent-lipid-receptor complexes cannot necessarily be regarded as homogeneous particles. Because lipids as well as receptors sequester detergent during the solubilization step, the ratio of detergent to membrane will determine how much detergent and lipid are bound around the receptor. The amount of bound lipid and detergent will change during the course of purification.

The solubilization procedure must be optimized to yield the highest extraction efficiency while maintaining the best stability of a given GPCR in solution. The effect of the systematic variation of detergent to membrane input can be monitored by performing radioligand binding assays and determining the total protein content. This allows the calculation of an experimental $B_{\max}$ value (nmol functional receptor/mg of protein) and comparison of that value with the theoretical value for specific binding of pure functional receptor (see also below). If no test for functionality is available, then good biochemical behavior such as a symmetric size exclusion chromatography profile can be used as an indicator for integrity.

The preparation of membranes prior to solubilization constitutes an initial purification step because soluble proteins are removed before receptor extraction and the ratio of target receptor to contaminants is therefore higher. However, receptors can also be enriched from total cell lysate rather than from solubilized membranes during the first purification step (see below).

Purification – General Considerations

Stability of GPCRs in Detergent Solution

Many GPCRs are not very stable in detergent solution (except the visual pigment rhodopsin as long as the receptor is kept in its non-signaling dark state (De Grip, 1982)). One possible explanation for this instability may be inherent structural flexibility i.e. the receptor can adopt several/many conformations in detergent solution, some of which may aggregate. Removal of lipid during the purification process may also cause destabilization. A number of measures have been taken to increase the stability of GPCRs in detergent solution. For example, the addition of an inverse agonist/antagonist ligand (Cherezov *et al.*, 2007; Hanson *et al.*, 2008; Jaakola *et al.*, 2008; Warne *et al.*, 2008) will cause the receptor to assume its non-signaling ground state which is generally considered more stable than the activated state(s) (see (Kobilka and Deupi, 2007)). Lipids or lipid-like substances such as cholesteryl hemisuccinate (Tucker and Grisshammer, 1996; Weiß and Grisshammer, 2002; Jaakola *et al.*, 2008) and glycerol (Tucker and Grisshammer, 1996) have been included throughout the purification to improve receptor stability. Site-directed mutagenesis has also been used to generate GPCRs with increased stability (Magnani *et al.*, 2008; Roth *et al.*, 2008; Sarkar *et al.*, 2008; Serrano-Vega *et al.*, 2008) and hence more tolerant to a wider range of detergents.

General Affinity Purification

The biochemical and pharmacological properties of GPCRs in detergent solution may arbitrarily be grouped into two categories. (i) Receptors which can be purified to homogeneity in functional form under optimized buffer conditions as assessed by radioligand binding assays and G-protein nucleotide exchange experiments (White *et al.*, 2007) are referred to as being functional. (ii) In some cases (Kobilka, 1995; White *et al.*, 2004) a detergent-soluble species has been observed which does not bind receptor-specific ligands. I refer to this latter species as non-functional, incorrectly folded (at least in respect to ligand recognition) but still detergent soluble.

Recombinant cloning techniques have made it easy to introduce general affinity tags at either the N-terminus or C-terminus of a given receptor. Examples are a Flag epitope tag at the receptor N-terminus for use with an M1 antibody affinity column (Kobilka, 1995) or poly-histidine tails at the receptor C-terminus for immobilized metal affinity chromatography (IMAC) (Kobilka, 1995; Grisshammer and Tucker, 1997; Hulme and Curtis, 1998; Klaassen *et al.*, 1999; Weiß and Grisshammer, 2002; Warne *et al.*, 2003; Yeliseev *et al.*, 2005; Hanson *et al.*, 2008; Jaakola *et al.*, 2008). An antibody column (1D4 antibody (Molday and MacKenzie, 1983)) recognizing the extreme C-terminus of bovine rhodopsin has been used for the single-step purification of rhodopsin (Oprian *et al.*, 1987; Reeves *et al.*, 1999) and of a β -adrenergic receptor with the 1D4 epitope tag fused to its C-terminus (Chelikani *et al.*, 2006).

The exact position of an affinity tag (i.e. well removed from the receptor transmembrane core or close to a transmembrane helix) determines whether binding of the receptor to the affinity resin must be done in batch or can be done in column mode. An affinity tag too close to the transmembrane core may be partially masked by the detergent belt around the receptor protein and hence will be less accessible to the affinity resin. Batch loading for a prolonged time will capture receptors efficiently in that case (Weiß and Grisshammer, 2002). Receptors with well exposed affinity tags can be loaded onto resin packed into a column with a short exposure time of the purification tag to the affinity resin. Batch purification procedures have to be performed manually, whereas column-loading procedures can be automated (White *et al.*, 2004). As general guideline, detergents with low critical micelle concentration (cmc) values form larger detergent belts around a membrane protein than detergents with high cmc values (see for example (Bamber *et al.*, 2006)) although the exact amount of protein-bound detergent will depend on the properties of the respective detergent tested. The accessibility of an affinity tag to the resin may therefore be reduced in low cmc detergents compared to high cmc detergents.

Many laboratories utilize IMAC as a first enrichment step. Several resin types are commercially available such as Ni^{2+} -NTA resin (Ni^{2+} -nitrilotriacetate, Qiagen), Talon resin (Co^{2+} -carboxymethylaspartate, Clontech), and IDA resin (iminodiacetate, Zn^{2+} , Ni^{2+} , Co^{2+} , GE Healthcare). However, the properties of the various IMAC resins are slightly different (Weiß and Grisshammer, 2002). For example, Ni^{2+} -NTA resin binds the target membrane protein tighter but also binds more contaminants compared to Talon resin. The receptor expression level (i.e. the ratio of the target receptor to contaminants) determines the efficiency of this first purification step; the higher the receptor expression level, the more efficient the first purification step. A purity of >90% has been achieved for a mutant β_2 -adrenergic receptor using a single IMAC step (Hanson *et al.*, 2008). In some cases, the binding capacity of IMAC resin has been found to be lower for receptors in detergent solution than that for soluble proteins (Weiß and Grisshammer, 2002; White *et al.*, 2004).

The time needed for purification should be kept to a minimum, as should the number of purification steps because of incurring protein losses at all stages.

Receptor-Specific Ligand Affinity Chromatography

After the enrichment of receptors by use of a general affinity tag, a second purification step may be required to isolate pure, functional receptor protein for a number of reasons. (i) Receptors from the first purification step are not yet pure and other contaminants are still present. For example, a major contaminant was removed from the adenosine A2a receptor by use of a XAC (xanthine amine congener, antagonist) column step (Weiß and Grisshammer, 2002). Likewise, the neurotensin receptor NTS1 was purified to homogeneity by use of a NT (neurotensin, agonist) column (White *et al.*, 2004) and an alprenolol (non-selective β -adrenergic receptor antagonist) Sepharose column was used for the purification of a β -adrenergic receptor (Warne *et al.*, 2003). If receptors, eluted from the general tag affinity resin, are a mixture of functional and non-functional, incorrectly folded receptors, then this subsequent receptor-specific ligand affinity chromatography step will also remove the non-functional receptor population. (ii) The use of one (or two subsequent) general affinity tag step(s) produces almost pure receptor which are however a mixture of functional and non-functional species. The use of general affinity tags will hence not resolve correctly folded from incorrectly folded but still detergent soluble protein. Incorrect folding of receptors may occur within the expression host, and/or may arise because of the instability of receptors in detergent solution. Application of a receptor-specific ligand affinity column will resolve functional from incorrectly folded receptor species. For example, an alprenolol column has been used for the isolation of functional β_2 -adrenergic receptor (Kobilka, 1995). (iii) A one step purification protocol led to a highly enriched preparation of a pituitary adenylate cyclase-activating polypeptide (PACAP) receptor by adding biotinylated peptide ligand (PACAP38) and avidin resin to the crude solubilized receptor (Ohtaki *et al.*, 1998).

Analysis of Detergent-Solubilized GPCRs

The amount of functional, detergent-solubilized receptor can be determined by radioligand binding experiments. Ideally, the radioligand should bind to the receptor with high affinity and should display a slow off-rate to avoid artifacts possibly arising because of non-equilibrium conditions during the process of separation of the ligand-receptor-detergent complex from free ligand. An outline into receptor binding studies is given in the book "Receptor Biochemistry – A Practical Approach" (Hulme (ed.), 1990). Radioligand binding analyses work best with labeled ligands which are hydrophilic and do not incorporate into empty detergent micelles. In contrast, hydrophobic ligands can insert into empty detergent micelles leading to high non-specific background signals.

It is recommended to determine the protein content by the Amido Black assay (Schaffner and Weissmann, 1973). Many methods for protein content determination are inaccurate in the presence of detergents and lipids. The Amido Black assay includes a precipitation step to remove detergents, lipids or other buffer components. Note that not all proteins bind dyes such as Amido Black equally well, meaning the protein concentration determinations may be biased if the dye-binding is different for the receptor compared to the reference protein (BSA).

From ligand-binding data and protein content analysis, one can calculate an experimental $B_{\max}$ value (nmol functional receptor/mg of protein) and compare this value with the

theoretical value for specific binding of pure functional receptor. Note that the theoretical $B_{\max}$ value is specific for each GPCR because it depends on its amino acid composition. Purification of functional receptors is achieved if the experimental value matches the theoretical $B_{\max}$ value.

Solubilization and purification of a recombinant neurotensin receptor NTS1

A more detailed description of the solubilization, purification and analysis of NTS1 has been described in the reference (White and Grisshammer, 2007) and is presented here in an abbreviated form. The purification to homogeneity of the NTS1 fusion protein (see below) is achieved by immobilized metal affinity chromatography (IMAC) followed by a NTS1-specific ligand column step.

A maltose-binding protein (MBP) fusion approach is used for the expression of functional, membrane-inserted NTS1 in *E. coli* (Grisshammer *et al.*, 1993). The expression plasmid encodes MBP with its signal peptide, followed by the receptor. After removal of the signal peptide by the *E. coli* leader peptidase, the NTS1 fusion protein MBP-T43NTR-TrxA-H10 (NTS1-624), used for purification, starts with the mature *E. coli* maltose-binding protein (MBP, Lys¹ to Thr³⁶⁶), followed by the N-terminally truncated rat neurotensin type I receptor NTS1 (T43NTR, Thr⁴³ to Tyr⁴²⁴) (Tanaka *et al.*, 1990), the *E. coli* thioredoxin (TrxA, Ser² to Ala¹⁰⁹) (the presence of TrxA increases expression, see (Tucker and Grisshammer, 1996)), and a decahistidine tag (H10) (Grisshammer and Tucker, 1997). Expression is driven from a weak promoter of the low-copy number expression plasmid at low temperature (22°C). This avoids the possible overloading of the *E. coli* translocation and membrane insertion machinery by the nascent receptor chain. Few protein molecules are produced at any given time but accumulation of correctly folded receptors is observed over 2 days. Purification of 10 milligrams of the NTS1 fusion protein typically requires 250 grams of *E. coli* wet cells which is equivalent to 50 liters of cell culture (White *et al.*, 2004). The growth of *E. coli* at this scale is most easily done by fermentation.

The expression levels of the NTS1 fusion protein in *E. coli* are moderate (i.e. the ratio of contaminants to target receptor is high). Hence this requires an optimized protocol for IMAC to enrich the receptor fusion protein efficiently. To accomplish this, NTS1 fusion proteins with C-terminal tails of ten histidine residues rather than six histidine residues (Grisshammer and Tucker, 1997) are used in combination with Ni²⁺-NTA resin. The tight binding to Ni²⁺-NTA resin of deca-histidine-tagged receptors allows stringent washing steps using imidazole at a concentration of 50 mM. Imidazole at this concentration eliminates binding to the Ni²⁺-NTA resin of most of the *E. coli* contaminants, but does not cause the elution of the fusion protein. This strategy not only results in efficient purification of receptors from crude membranes, but works well for the purification from total cell lysate (Grisshammer and Tucker, 1997).

The apparent affinity of NT for NTS1 is reduced in the presence of the high concentrations of sodium ions and imidazole in the NiB buffer (see below) (Grisshammer *et al.*, 1999) precluding the direct binding of functional receptors in the Ni²⁺-NTA column eluate onto the NT column. The concentration of NaCl and imidazole must therefore be reduced from

200 mM to 70 mM by dilution with buffer to allow binding of functional NTS1 to the NT column. Because binding of NTS1 to NT is NaCl sensitive, receptors can be efficiently eluted from the NT column with NaCl at high concentration.

Solubilization of the NTS1 Fusion Protein

1. All steps are performed at 4°C or on ice unless stated otherwise.
2. At room temperature, crush 250 grams of frozen cell paste between plastic sheets with a hammer to obtain small pieces. Place cells into a Waring blender.
3. Add 500 ml of cold 2× solubilization buffer (100 mM Tris-HCl pH 7.4, 60% v/v glycerol, 400 mM NaCl) to the cells. Operate the Waring blender until all cells are suspended.
4. Transfer the cell suspension into a beaker with a magnetic stir bar. It is difficult to determine the volume at this stage because of the air introduced by the Waring blender into the suspension. The final volume is therefore adjusted in step 11.
5. While stirring, add 1 ml of protease inhibitor stock solutions (phenyl methyl sulphonyl fluoride, PMSF, at 70 mg/ml in ethanol; leupeptin at 1 mg/ml in H₂O; pepstatin A at 1.4 mg/ml in methanol), 5 ml of 1 M MgCl₂ (final concentration of 5 mM), 0.6 ml of DNaseI solution (10 mg/ml, Sigma D-4527), and 50 ml of cold H₂O.
6. While stirring, add dropwise 100 ml of a CHAPS/CHS stock solution (6% w/v (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate/1.2% w/v cholesteryl hemisuccinate Tris salt in H₂O). CHS is not water soluble on its own and needs to be dissolved in CHAPS.
7. While stirring, add dropwise 100 ml of a DDM stock solution (10% w/v n-dodecyl-β-D-maltoside in H₂O).
8. Continue to stir for 15 min.
9. Sonicate for 33 min (8 sec/gram of cells), 1 sec on, 2 sec off, level 4 (Misonix sonicator 3000, ½ inch flat tip). Keep the sample in an ice/water bath to avoid local heating during sonication. This mild sonication step enhances the receptor solubilization efficiency.
10. Add an additional 1 ml of each protease inhibitor stock.
11. Determine volume and add cold H₂O dropwise under stirring to a final volume of 1 L.
12. Stir sample for an additional 30 min.
13. Ultracentrifuge sample for one hour (Beckman 45Ti rotors or equivalent, at 45,000 rpm (235,000 × g at r_{max})).
14. Retrieve the solubilized receptors (supernatant).
15. Add an imidazole stock solution (2 M, adjusted to pH 7.4 with HCl) dropwise while stirring (final concentration of 50 mM). Pass sample through 0.22 μm filter.

The supernatant is now ready for purification of receptors by IMAC followed by a neurotensin affinity column.

Purification of the NTS1 Fusion Protein by Immobilized Metal Affinity Chromatography

The purification is carried out in the cold room using an Äkta Purifier (GE Healthcare) chromatography system in a fully automated two-column mode; for details see (White *et al.*, 2004; White and Grisshammer, 2007). The Purifier is equipped with air sensors, a sample pump P950, a modified injection valve, a 100-ml Ni²⁺-NTA column, a 20-ml NT column, and a fraction collector Frac950. However, all purification steps can also be performed in a simpler setting. The IMAC column can be processed first, and the Ni²⁺-NTA column eluate is then diluted (see below) and loaded onto the NT column.

1. Equilibrate a 100-ml Ni²⁺-NTA Superflow (Qiagen) column (XK50, GE Healthcare) with NiA buffer (50 mM Tris-HCl pH 7.4, 30% v/v glycerol, 50 mM imidazole, 200 mM NaCl, 0.5% w/v CHAPS/0.1% w/v CHS, 0.1% w/v DDM).
2. Load the supernatant at a flow rate of 2 ml/min onto the Ni²⁺-NTA column using the sample pump equipped with an air sensor to detect the end of loading. Collect the Ni²⁺-NTA column flow through for analysis.
3. After loading is completed, wash the Ni²⁺-NTA column with 15 column volumes of buffer NiA at 2 ml/min.
4. For elution, pass 4 column volumes of buffer NiB (buffer NiA but with 200 mM imidazole) at 2 ml/min over the Ni²⁺-NTA column.
5. The total volume of fractions containing the eluted NTS1 fusion protein is ~120 ml.

Purification of the NTS1 Fusion Protein by a Neurotensin Column

Neurotensin has nano-molar affinity for its receptor in the presence of detergents; therefore, this property can be used for an efficient affinity purification step (Tucker and Grisshammer, 1996; Grisshammer *et al.*, 1999). The NT resin is based on N-terminally biotinylated NT (biotin- β Ala- β Ala-Gln-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH), bound to tetrameric avidin resin. Avidin has a high isoelectric point and must be succinylated to reduce the binding of *E. coli* proteins to the affinity matrix (Tucker and Grisshammer, 1996). Biotinylated NT is made by solid-phase peptide synthesis.

1. Equilibrate a 20-ml NT column (XK26) with NT70 buffer (50 mM Tris-HCl pH 7.4, 30% v/v glycerol, 1 mM EDTA, 70 mM NaCl, 0.5% w/v CHAPS/0.1% w/v CHS, 0.1% w/v DDM).
2. Dilute the Ni²⁺-NTA column eluate with buffer NT0 (50 mM Tris-HCl pH 7.4, 30% v/v glycerol, 1 mM EDTA, 0.5% w/v CHAPS/0.1% w/v CHS, 0.1% w/v DDM) to reduce the imidazole and NaCl concentrations to 70 mM, respectively (see above). The dilution step is performed by the Äkta Purifier (White *et al.*, 2004), but can also be done manually.
3. Load the diluted Ni²⁺-NTA column eluate onto the NT column at a flow rate of 0.4 ml/min. Collect the NT column flow through for analysis.

4. Wash the NT column at 0.7 ml/min with 8 column volumes of buffer NT70 (see above).
5. Elute receptors at a flow rate of 0.5 ml/min with buffer NT1K (NT0 buffer with 1 M NaCl).
6. After the purification is completed, wash the NT column extensively with buffer A3 (50 mM Tris-HCl pH 7.4, 1 mM EDTA, 1 M NaCl) followed by buffer B3 (50 mM Tris-HCl pH 7.4, 1 mM EDTA, 3 mM NaN₃). Buffer B3 is used as storage buffer.

Analysis of purified NTS1

The quality of the NTS1 preparation can be assessed by radioligand binding assay using tritium-labeled neurotensin ([³H]NT). [³H]NT is very hydrophilic and not 'sticky', resulting in low background binding. The detergent-solubilized NTS1 fusion protein binds the agonist with nano-molar affinity. The off-rates are slow, which allows the separation of the ligand-receptor-detergent complex from free ligand (which does not incorporate into free detergent micelles) by centrifugation-assisted gel filtration (White *et al.*, 2004). The size difference between [³H]NT and the ligand-receptor-detergent complex is large enough to avoid 'leakage' of free [³H]NT. For routine analyses, the [³H]NT concentration is set to 2 nM. With some assumptions, one can correct for fractional occupancy (the law of mass action predicts that the fractional receptor occupancy at equilibrium is a function of the ligand concentration: fractional occupancy = [ligand]/([ligand] + K_D)). A theoretical value for specific binding (B_{max}) of 10.4 nmol/mg is calculated for the NTS1-624 fusion protein (molecular mass of 96.5 kDa), assuming one ligand-binding site per receptor molecule. The protein content is determined by the Amido Black assay (Schaffner and Weissmann, 1973).

Starting from 250 grams of wet *E. coli* cells, the following results can be anticipated: Supernatant (~930 ml, ~16 mg protein/ml, ~0.2 nmol NTS1/ml, ~10–12 pmol NTS1/mg protein); Ni²⁺-NTA column eluate (~120 ml, ~0.25 mg protein/ml, ~1.2 nmol NTS1/ml, ~5 nmol NTS1/mg protein); neurotensin column eluate (~14 ml, ~0.7 mg protein/ml, ~7 nmol NTS1/ml, ~10 nmol NTS1/mg protein).

Conclusions

The NTS1 receptor is expressed in functional form in *E. coli* as a fusion protein. Receptors are solubilized by a detergent mixture from total cell extract (rather than from a membrane preparation) and are enriched by immobilized metal affinity chromatography. Contaminants present in the Ni²⁺-NTA column eluate are removed by the use of a subsequent neurotensin column. This above purification protocol is simple and robust and yields pure, functional NTS1 fusion protein.

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FIG. 1.

Purification of the neurotensin receptor NTS1. The fusion protein NTS1-624 was purified on a 100-ml Ni^{2+} -NTA column followed by a 20-ml NT column, starting from 250 grams of *E. coli* cells. The progress of purification was monitored by SDS-PAGE (NuPAGE 4-12 % Bis-Tris gel, Invitrogen, 1 $\times$ MES buffer) and Coomassie R-250 staining. Lane M: Novagen Perfect Protein Marker (15–150 kDa); lane 1: 10 μg of supernatant; lane 2: 10 μg of Ni^{2+} -NTA column flow through; lane 3: 5 μg of Ni^{2+} -NTA column eluate; lane 4: 10 μg of NT column flow through; lane 5: 5 μg of NT column eluate. The incorrectly folded receptors in

the NT column flow through are initially detergent soluble but aggregate over time (R. Grisshammer, unpublished results). Reprinted from 'Automated large-scale purification of a G protein-coupled receptor for neurotensin' by White *et al.*, FEBS Letters 564, 289–293, 2004.

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