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Overexpression and Purification of Human Calcitonin Gene Related Peptide - Receptor Component Protein (CGRP-RCP) in *Escherichia coli*

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Abstract

Calcitonin gene-related peptide (CGRP) is a neuropeptide secreted by the central and peripheral nervous system nerves that has important physiological functions such as vasodilation, cardiotonic actions, metabolic and pro-inflammatory effects. The CGRP receptor is unique among G-protein coupled receptors in that a functional CGRP receptor consists of at least three proteins: Calcitonin Like Receptor (CLR), Receptor Activity Modifying Protein (RAMP1) and Receptor Component Protein (RCP). RCP is a required factor in CGRP-mediated signal transduction and it couples the CGRP receptor to the signal transduction pathway. Here we describe methods to overexpress and purify RCP for structure-function studies. Human RCP was cloned and overexpressed with a poly-histidine tag and as a Maltose Binding Protein (MBP) fusion in *Escherichia coli* using commercially available expression vectors. While his-tagged RCP is prone to aggregation, solubility is improved when RCP is expressed as a MBP fusion. Expression and purification procedures for these constructs are described. Results from these studies will facilitate structural analysis of human RCP, and allow further understanding of RCP function.

Keywords

Calcitonin Gene Related Peptide Receptor Component Protein (CGRP RCP); Maltose Binding Protein Fusion; Recombinant Protein; Expression; Purification; Solubility

Introduction

Calcitonin gene-related peptide (CGRP) is a 37-amino acid carboxyl-amidated neuropeptide secreted by nerves of the central and peripheral nervous system [1]. CGRP is found throughout the cardiovascular system and in all regions of the heart [2]. It is one of the most potent vasodilators known and can affect the force and rate of heart beat [3,4]. Several studies have shown the importance and relevance of CGRP and its receptor to the heart [5,6]. Springer *et al.*, 2003 [7], discuss a strategy to target CGRP and effect nerve modulation and inflammation. Moreover, CGRP release is involved in neurogenic vasodilatation, neurogenic inflammation

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and migraine [8]. Recently, the non-peptide CGRP antagonist BIB4096N has been used successfully in clinical trials to alleviate migraine symptoms [9,10].

CGRP binding results in increased intracellular cAMP levels and a candidate G-protein-coupled receptor has been identified – the calcitonin like receptor (CLR). It is becoming clear that many receptors that initiate signal transduction by interacting with G-proteins do not function in isolation, but require accessory proteins for function. Some of these accessory proteins are chaperones [11–14]. One such protein, the CGRP-receptor component protein (RCP) was discovered during expression-cloning experiments targeting the CGRP receptor [15]. RCP is a 148 amino acid hydrophilic protein which is required for CGRP receptor activation in oocytes, as well as in cells and tissues that express CGRP binding sites [16]. It is present in vasculature and the heart, and co-localizes with CGRP in cerebellum, spinal cord and cochlea [15–18]. RCP has been cloned from guinea pig, mouse, rat, and human, and there is 80% identity at the protein level between RCP from these species [15,19–21] (Fig. 1).

Antisense studies have suggested that RCP couples the CGRP receptor to downstream effectors, and co-immunoprecipitation studies have shown that CLR and RCP form a complex in cell culture and tissues [16,22]. Furthermore, expression of RCP correlates with CGRP receptor activation *in vivo*, [19,23], and RCP expression is modulated by inflammation, neuropathic pain, and by the application of antagonist [24]. Thus, RCP represents a dynamic regulator of G protein-coupled signal transduction, and implies an additional level of receptor regulation unique to the CGRP system. CGRP signals through CLR, and has been previously shown to require the accessory protein RAMP 1 (receptor activity modifying protein) for function [25]. These studies have led to a model where the functional CGRP receptor consists of at least three proteins: 1) Receptor, CLR, 2) Chaperone protein, RAMP1 and 3) RCP that couples the receptor to the cellular signal transduction pathway (Fig. 2).

Interestingly, Johnson *et al.*, 2005 [26], provides evidence for conservation of CGRP signaling. They show that *Drosophila* neuropeptide DH_{31} (CGRP) is a potent ligand for CG17415 (orphan G protein-coupled receptor related to the calcitonin receptor-like receptor) and that CLR accessory proteins are also required for this activity.

Moreover, a study by Siaut *et al.*, 2003 [27] points to a possible dual role for RCP. They determined that C17 (an essential subunit of yeast RNA polymerase) interacts with Brf1 (a component of TFIIIB), which suggests a role for C17 in initiation of transcription. RCP is the mammalian orthologue of C17, and human RCP could functionally replace its orthologue in yeast. These authors suggest a dual role for RCP in Pol III transcription, as well as in signal transduction; this would represent an unusual example of functional divergence. Siaut *et al.*, 2003 [27] carried out sequence analysis and molecular modeling, to show that the C17/C25 heterodimer could likely adopt a structure similar to that of the archaeal RpoF/RpoE (Pol III) counterpart of the Rpb4/Rpb7 (Pol II) complex.

The primary amino acid sequence of RCP does not suggest how the protein enables CLR signaling or how it might aid transcription. The RCP primary sequence does not contain any known protein motifs, and there are also no 3D structures available for RCP. Structure-function studies are thus critical to understand this protein. As a first step towards structural studies, we need purified RCP. We report here the methods for overexpression and purification of full-length human RCP in *E. coli* tagged with either poly-histidine or maltose binding protein (MBP), as well as, characterization of the proteins using dynamic light scattering (DLS) and circular dichroism (CD) spectroscopy.

Materials and Methods

Materials

pET28b (Promega Corp.) and pMALc2E (New England Biolabs Inc.) were used as the expression vectors. *E. coli* strain BL21(DE3) (Novagen Inc.) was used as the host. PCR primers were purchased from Sigma-Genosys.

Methods

Construction of pET28b.hRCP—Human RCP (GenBank accession number AF073792) was cloned by degenerate RT-PCR, using methods described previously [20,21]. hRCP was then inserted between the Nde I and Hind III sites in pET28b vector to yield a full-length N-terminal 6XHis tagged hRCP. The sequence and orientation of the insert were confirmed by DNA sequencing.

Construction of pMALc2E.hRCP—hRCP was subcloned between the Eco RI and Hind III sites in the pMALc2E vector to yield a full-length N-terminal maltose binding protein (MBP) tagged hRCP. The primers used for PCR were HindIIIhRCP (‘5- AAA AAG CTT CTA TGC TGG GTC CTC TTC GTC CAT TGC CA -3’) and hRCPEcoRI (‘5- AAA GAA TTC ATG GAA GTG AAG GAT GCC AAT TCT GCG -3’). The sequence and orientation of the insert were confirmed by DNA sequencing.

Construction of pMALc2E.shRCP—A vector was constructed for expressing MBP-hRCP fusion protein in which the linker between the MBP and hRCP is reduced to 3 alanines instead of the 25 residues encoded in the original vector. The primers for PCR, MBPshRCPforward (‘5- GAA AGC GGC CGC AAT GGA AGT GAA GGA TGC CAAT TCT G -3’) and MBPshRCPreverse (‘5- GAA AGC GGC CGC AGT CTG CGC GTC TTT CAG GGC TTC ATC GAC -3’), contained flanking Not I sites. Not I restriction enzyme was chosen for this purpose since its recognition sequence codes for alanines. After PCR using the pMALc2E.hRCP as the template, the product was digested with Not I. The digested product was self ligated and then transformed into competent cells. The sequence of the MBP-shRCP gene fusion containing the three alanines and the full RCP gene were confirmed by DNA sequencing.

Expression and purification of Histidine tagged hRCP—The recombinant plasmid pET28b.hRCP was transformed into competent *E. coli* BL21(DE3). The bacteria was cultured in LB medium (Difco™ LB Broth, Miller Luria-Bertani) with vigorous shaking (210 rpm) at 37 °C to an optical density of 0.8 measured at 595 nm. Then, the culture was transferred to 22 °C and 1 mM isopropyl-β-D thiogalacto-pyranoside (IPTG) was added to induce the expression of the recombinant protein at 22 °C for 16 h. After induction, the cultures were centrifuged at 5000*g* for 10 min at 4 °C. Fifteen grams (wet weight) of pellet was resuspended in 35 ml of Buffer Ni-A (20 mM Tris–HCl, 300 mM NaCl, and 20 mM Imidazole, pH 8.0). Cell lysis was done using FrenchR Pressure Cell Press (SLM Instruments, Inc.). Before cell lysis, one Complete Mini EDTA-free protease inhibitor cocktail tablet (Roche Inc.) and 0.1 mM Phenylmethylsulphonylfluoride (PMSF) was added to the resuspended cells.

The lysate was centrifuged at 20,000*g* for 30 min at 4 °C and Polymin P was added to 0.3% by volume. The solution was stirred on ice for 15 min and centrifuged at 10,000*g* for 10 min at 4 °C. An ammonium sulphate (AMS) cut of 0.97 M – 1.5 M was applied to the sample. First, AMS was added to the polymin P supernatant to a concentration of 0.97 M and the sample was stirred on ice for 30 min. The solution was centrifuged at 20,000*g* and AMS was added to the supernatant, to a final concentration of 1.5 M. After 30 minutes on ice, the precipitated proteins were recovered by centrifugation at 20,000*g*.

The pellet obtained from the protein extraction step was dissolved in Buffer Ni-A, and loaded on an immobilized metal affinity column (IMAC) with Poros®MC-20 beads (Boehringer Mannheim Inc.) which was pre-equilibrated with Buffer Ni-A. The column was washed with Buffer Ni-A and bound protein was eluted using an imidazole gradient from 20 to 500 mM on an AKTA FPLC chromatography system (GE Healthcare Inc.).

Protein elutions were monitored at 280 nm and the resulting fractions were analyzed by SDS electrophoresis on Gradient 8–25 PhastGel™ (GE Healthcare Inc.). The eluted fractions were dialyzed against Buffer MQ-A (20 mM Tris–HCl, 2 mM EDTA, 1 mM DTT, 275 mM NaCl, pH 8.0). The sample was applied to a MonoQ®HR anion-exchange chromatography column (GE Healthcare Inc.) pre-equilibrated with Buffer MQ-A. Elution was performed using a gradient to 1M NaCl. Relevant fractions were concentrated by centrifuging at 4000*g* using an AmiconUltra centrifugal filter (Millipore Inc.) to 2.5 ml, and applied on a Superdex S-200 size exclusion column (GE Healthcare Inc.) pre-equilibrated with the Buffer S200 (20 mM Tris–HCl, 2 mM EDTA, 1 mM DTT, 350 mM NaCl, 10% glycerol, pH 8.0). The S-200 column was run at a flow rate of 1 ml/min. Protein elutions were monitored at 280 nm and the resulting fractions were analyzed by electrophoresis on Gradient 8–25 PhastGel™ (GE Healthcare Inc.). Protein bands were quantified using ImageQuant TL v2005 (GE Healthcare Inc.). Purified His tagged hRCP was concentrated to 10 mg/ml as measured by absorbance at 280 nm and the protein was stored at −80 °C after flash freezing in liquid nitrogen.

Expression and Purification of Maltose Binding Protein tagged hRCP—The recombinant plasmid pMALc2E.hRCP was transformed into competent *E. coli* BL21(DE3). The bacteria was cultured in 0.2 % glucose LB medium (Difco™ LB Broth, Miller Luria-Bertani) with vigorous shaking (210 rpm) at 37 °C to an optical density of 0.8 measured at 595 nm. Then, the culture was transferred to 25 °C and 1 mM IPTG was added to induce expression of the recombinant protein at 25 °C for 16 h. After induction, the cultures were centrifuged at 5000*g* for 10 min at 4 °C.

Three grams (wet weight) of pellet was resuspended in 35ml Buffer M-A (2 0mM Tris–HCl, 300 mM NaCl, 1 mM DTT and 1 mM EDTA, pH 7.4). Cell lysis was done using EmulsiFlex-C3 (Avestin Inc.). Before cell lysis, one Complete Mini EDTA-free protease inhibitor cocktail tablet (Roche Inc.) and 0.1 mM PMSF was added to the resuspended cells.

The lysate was centrifuged at 20,000*g* for 30 min at 4 °C. The supernatant was diluted to a final concentration of 2 mg/ml. For purification of MBP-hRCP, the cleared lysate was loaded onto an amylose column (New England Biolabs Inc.) and the fractions were eluted using 20 mM maltose. The pooled fractions were then loaded on a Superdex S-200 size exclusion column (GE Healthcare Inc.) pre-equilibrated with the Buffer M-B (20 mM Tris–HCl, 300 mM NaCl, 2 mM EDTA, 1 mM DTT and 10 % glycerol, pH 7.4) and separated at a flow rate of 1 ml/min. Purified MBP-hRCP was concentrated to 16 mg/ml as measured by absorbance at 280 nm and the protein was stored at −80 °C after flash freezing in liquid nitrogen.

To test RCP solubility in absence of the MBP tag, purified MBP-hRCP protein was cleaved using recombinant enterokinase (Novagen Inc.). 250 μg of MBP-hRCP was cleaved overnight using 0.01 units of enterokinase in 500 μl of cleavage buffer. The resulting sample was loaded on a Superdex S-200 size exclusion column (GE Healthcare Inc.) for characterization.

Expression and Purification of Maltose Binding Protein tagged hRCP with a short linker—pMALc2E.shRCP, was transformed into BL21(DE3) cells and induced at 25 °C overnight, with 1 mM IPTG. The expressed MBP-shRCP was purified using the method previously optimized for purification of MBP-hRCP (see above). The MBP-shRCP was

concentrated to 10 mg/ml as measured by absorbance at 280 nm, and the protein was stored in −80 °C after flash freezing in liquid nitrogen.

Antibodies and Western Blotting—RCP antibody (1065) is a chicken polyclonal antibody raised against a peptide with the sequence EQIEALLHTVT [16,17,24] which corresponds to residues 111–121 of RCP. 5 μg of purified recombinant RCP and 40 μg NIH 3T3 cell lysate were resolved by 15% SDS-PAGE, transferred to polyvinylidene difluoride membrane (Bio-Rad Inc.), and immunoblotted with antibodies directed against RCP (1065) diluted to 1:1000. The membrane were then washed with phosphate buffered saline plus 1% milk with 0.04% Tween 20 (PBS-T) and incubated with 1:50,000 dilution of donkey anti-chick secondary antibody conjugated to horseradish peroxidase (GE Healthcare Inc.) for 30 min. The membranes were washed with PBS-T, incubated in SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL) for 5 min, and exposed to film.

Dynamic Light Scattering—Dynamic light scattering (DynaPro, Wyatt Technology Inc.) was used to characterize the oligomerization state of RCP and to assess potential aggregation. Purified proteins were diluted to 1 mg/ml in a buffer containing 150 mM NaCl 10 mM Tris-HCl pH 7.4, 0.5 mM DTT, 1 mM EDTA and 10% glycerol. Analysis was done in 4 replicates following manufacturers recommendations (Wyatt Technology Inc.).

Circular Dichroism Spectroscopy—Circular dichroism measurements were performed using Jasco J-715 spectrapolarimeter (Jasco, Tokyo, Japan). MBP-hRCP and MBP-shRCP were dialysed against the CD buffer overnight (150 mM NaFl, 10 mM Sodium Phosphate pH 7) and diluted to 0.5 mg/ml before data collection. Data points were collected from 200 to 250 nm at 25 °C using a 0.1 cm cuvette. Each spectrum was the average of 10 scans. The CD spectra were obtained in milli-degrees and converted to molar ellipticity.

Results and Discussion

Histidine Tagged RCP

The expression of his-tagged recombinant human RCP in *E. coli* was monitored by SDS-PAGE. The results showed that optimal hRCP (20 kDa) expression was obtained with BL21 (DE3) cells grown overnight at 22 °C after induction with 1 mM IPTG (Fig. 3). An initial polymin P step was used to remove nucleic acids and nucleic acid binding proteins, followed by a step-wise AMS precipitation step. A sequential chromatography approach was used to further purify RCP. The pellet obtained from the protein extraction step was first applied to an immobilized metal chelate affinity column. RCP eluted at approximately 108 mM Imidazole. Pooled fractions were next applied on an anion exchange MonoQ column, and RCP eluted at approximately 360 mM NaCl. Finally, fractions from the anion exchange column were applied on a Superdex S-200 sizing column for further purification. (Fig. 3; Table I). Based on the S-200 elution profile, his tagged hRCP appears to be monomeric. The final His tagged hRCP yield by this procedure was about 4 mg from 15 g of wet cell paste.

While some of the recombinant RCP expressed in this manner is soluble, a large fraction (approximately 2/3) of the protein is present in the insoluble cell pellet (Fig. 3). To investigate the feasibility of using the insoluble pellet for protein production, we also tested a refolding protocol for RCP. For this purpose, we adapted the ‘on column refolding’ approach [28,29]. This approach yielded higher amounts of protein with good purity (data not shown). Both renatured RCP, as well as RCP purified from the soluble fraction, displayed immunoreactivity with an antibody against a peptide derived from the RCP amino acid sequence as shown by Western blotting (Fig. 4).

Unfortunately, RCP purified from both the soluble and the insoluble fractions had a tendency to aggregate (as determined by DLS, data not shown) which can interfere with protein crystallization. To overcome this problem, we tried several buffer conditions, including high salt (1–2 M NaCl) and sucrose (1 M) [30], but no improvement was seen in the tendency of RCP to aggregate.

Solubility tags

To further increase the yield of soluble RCP and decrease aggregation, we tested hRCP in fusion with different solubility tags, including thioredoxin (Trx) and MBP. For this, hRCP was cloned into the pET32b vector (Promega Corp.) to express TRX-hRCP, and the pMALc2E vector (New England Biolabs Inc.) was used to express MBP-hRCP. Small scale expression trials with the thioredoxin tag did not show any improvement in the solubility of RCP (data not shown). On the other hand, the use of the MBP tag increased solubility of RCP significantly. The best expression of MBP-hRCP (62 kDa) was achieved by using BL21(DE3) cells at 25 ° C, induced overnight with 1 mM IPTG. For purification of MBP-hRCP, the cleared lysate was loaded on an amylose column and bound proteins were eluted using 20 mM maltose. Most MBP-hRCP appeared in the first six fractions; these were pooled, concentrated, and loaded on a size exclusion column for further purification (Fig. 5; Table 2). Based on the S-200 elution profile, MBP-hRCP appears to be monomeric. The protein yield was about 6 mg from 3 g wet cell paste.

One of our aims is to crystallize RCP for structure studies. There are several examples of MBP fusion proteins used for crystallization [31–33]. However, mobility of domains linked by long linkers can adversely affect crystal formation. Shortening or removing the linker in fusion proteins has been used by several researchers to improve protein crystallization [33]. We pursued a similar strategy, by designing a short linker with three alanines for our MBP-hRCP construct (MBP-shRCP). The best expression of MBP-shRCP (approx. 60 kDa) was obtained in BL21(DE3) cells induced overnight with 1 mM IPTG at 25 °C. The expressed MBP-shRCP was purified using the same procedure as for MBP-hRCP (Fig. 6;Table 3). MBP-shRCP was monomeric as determined by size exclusion chromatography. The MBP-shRCP yield was about 4 mg from 5 g of wet cell paste. Crystallization trials are underway with the purified MBP-shRCP using hanging drop vapor diffusion technique with sparse matrix screening methods [34].

Dynamic Light Scattering and Circular Dichroism

DLS was used to further characterize MBP-shRCP. DLS measures fluctuations in the intensity of light scattered by a macromolecular solution which can be related to its hydrodynamic radius (Rh). DLS is also an excellent technique to monitor protein aggregation, and can be a valuable diagnostic tool for crystallization studies. Proteins with monomodal size distributions have higher probability of producing crystals [35]. The DLS analysis of MBP-shRCP (Fig. 7) shows a monomodal distribution of the protein in solution, indicating its suitability for crystallization trials.

We used circular dichroism spectroscopy to monitor proper folding of MBP-hRCP and MBP-shRCP. The spectra (Fig. 8) for both the constructs indicate well-folded proteins with a mixture of α-helix and β-sheet structural elements [36].

Conclusions

RCP represents a new class of signal transduction factor that is required for coupling CLR to the cellular signal transduction machinery [16]. While RCP expression has been observed in all mammalian tissues, extraction and purification of endogenous RCP is difficult and results

in very low yields. We describe here the use of a bacterial expression system for large-scale human RCP production.

RCP is a membrane associated cytoplasmic protein and is prone to aggregation, possibly reflected in the formation of dimers and multimers observed in lysates from tissue and cell culture [16,22]. When expressed in *E. coli* with a histidine tag, RCP is mostly insoluble; RCP purified from the soluble fraction is also susceptible to aggregation. We show that a MBP tag can be used to overcome these problems and obtain protein suitable for structure-function studies. Interestingly, the MBP tag appears to be essential for keeping RCP in a soluble form. When the MBP tag was removed using recombinant enterokinase, RCP tends to aggregrate into large complexes that come out in the void volume when applied on a size exclusion column (data not shown). Our approach of using an MBP tag yields milligram quantities of human RCP, and is the first example of high level RCP protein purification from a bacterial expression system.

Expression of mouse RCP for structural studies has also been attempted by the Center for Eukaryotic Structural Genomics (CESG), using a cell-free system [37,38]. While RCP was produced by this approach, the protein preparation was poorly defined as indicated by uninterpretable NMR spectra (J. L. Markley, personal communication).

Results described here for the MBP fusion hRCP should facilitate detailed structure-function studies of this protein. RCP plays an important role in the CGRP Receptor complex, which is involved in a number of physiological functions. Studies of RCP should shed light on the mechanism of CGRP receptor activation. The wide spectrum of pathways that involve CGRP have also evoked considerable interest in the development of drugs which can modulate its actions. In this respect, determination of the molecular structure of RCP and understanding its interactions with the CGRP receptor partners will help development of new ligands for the CGRP receptor.

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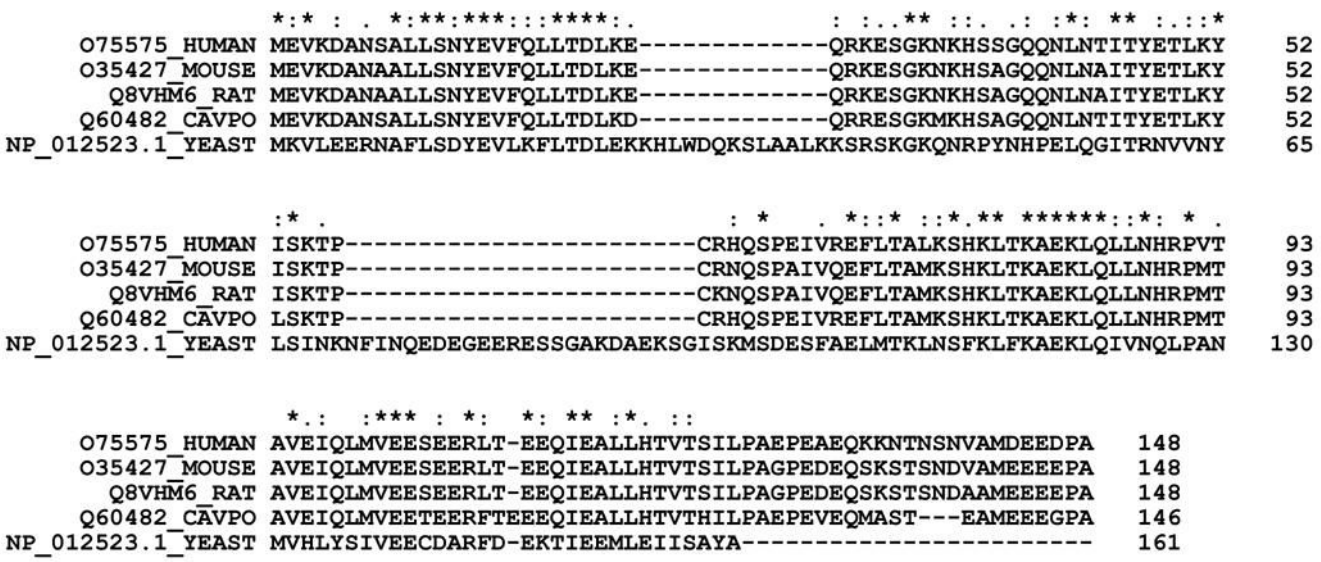


Figure 1.

Alignment of RCP protein sequences from selected species. Sequences are from the EXPASY/SWISS-PROT protein sequence database. The accession number precees the common name of the species in the alignment; O75575_HUMAN from *Homo sapiens*, O35427_MOUSE from *Mus musculus*, Q8VHM6_RAT from *Rattus norvegicus*, Q60482_CAVPO from *Cavia porcellus* and NP_012523.1_YEAST from *Saccharomyces cerevisiae*. The sequence alignment was generated using Clustal X (1.81). "*" indicates positions which have a single, fully conserved residue. ":" indicates strong conservation of residue type, while "." indicates weaker conservation.

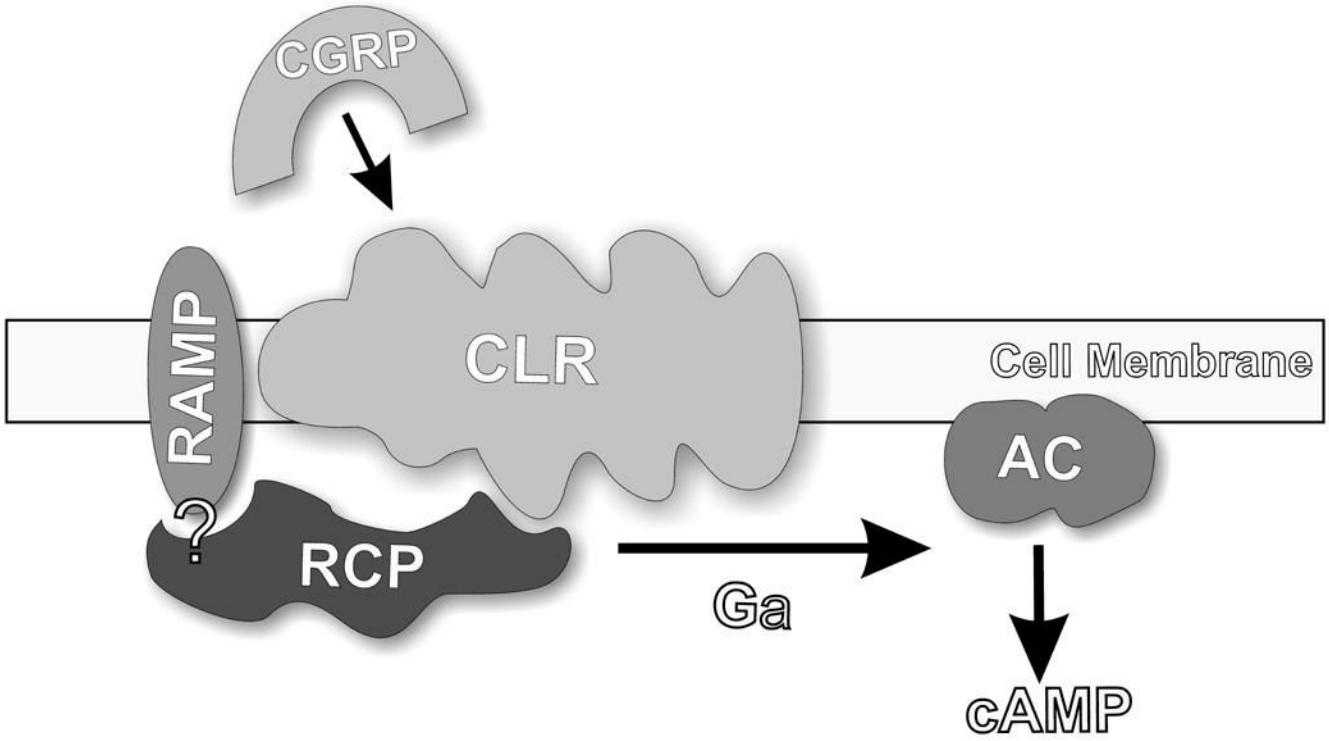


Figure 2 .
Model for the CGRP receptor complex [16,22]. CGRP, calcitonin gene related peptide. CLR, calcitonin like receptor. RAMP1, receptor activity modifying protein. RCP, receptor component protein. AC, adenylyl cyclase.

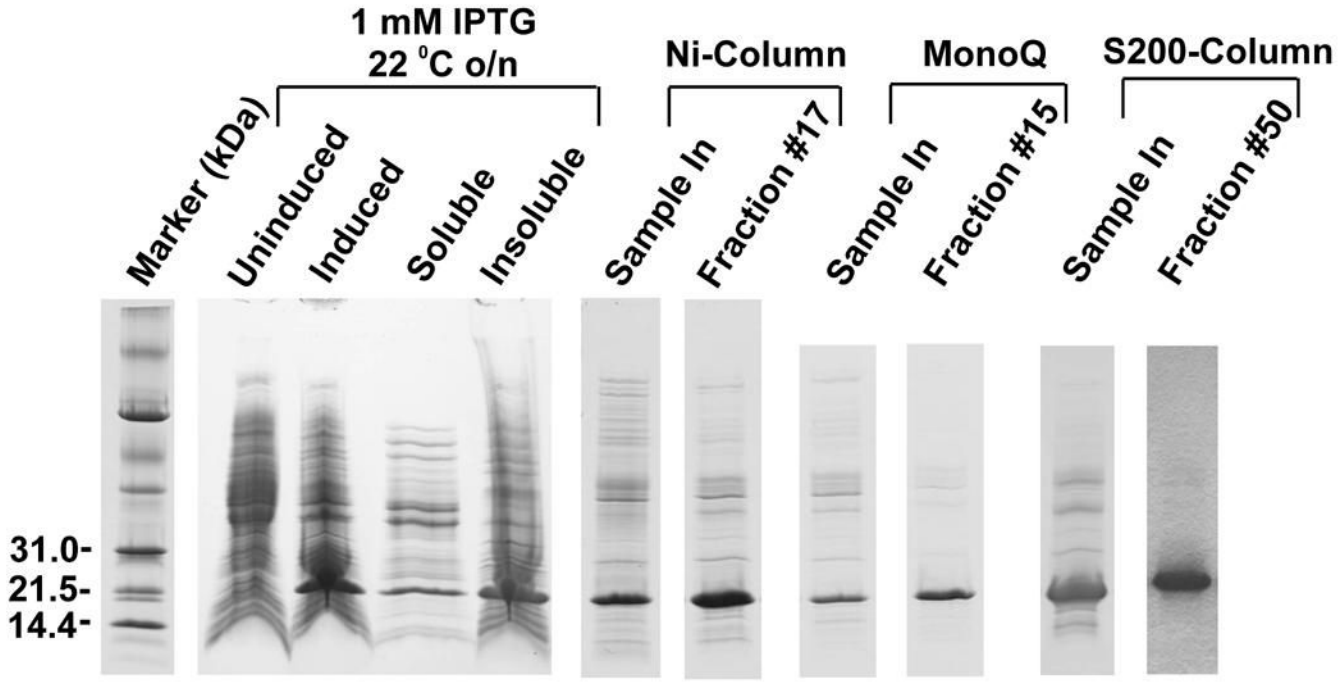


Figure 3.

Purification of hRCP. SDS-PAGE analysis of the expression and purification scheme for histidine tagged hRCP from soluble BL21(DE3) cell extract. A metal-chelation step was followed by anion exchange and size exclusion steps. All gels (8–25% Phast gels) were run under identical conditions on the Phast Gel system (GE Healthcare Inc.). Only fractions containing the peak are shown. (Sample In: Sample applied on the given column.)

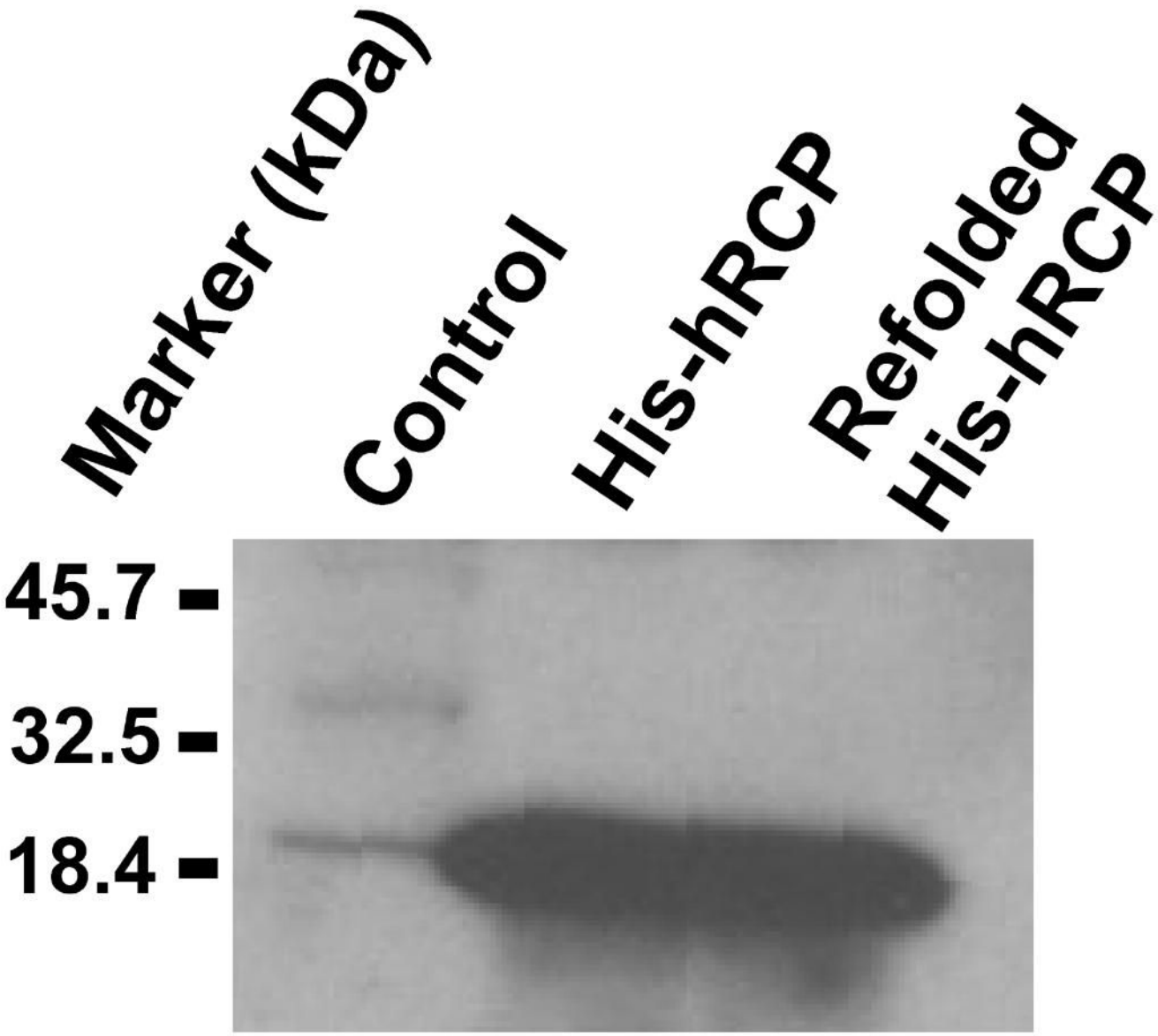


Figure 4.
Detection of RCP by Western blot. Five μg of purified recombinant RCP and 40 μg NIH 3T3 cell lysate were resolved by 15% SDS-PAGE, and immunoblotted with antibodies directed against a peptide derived from the RCP amino acid sequence (1065).

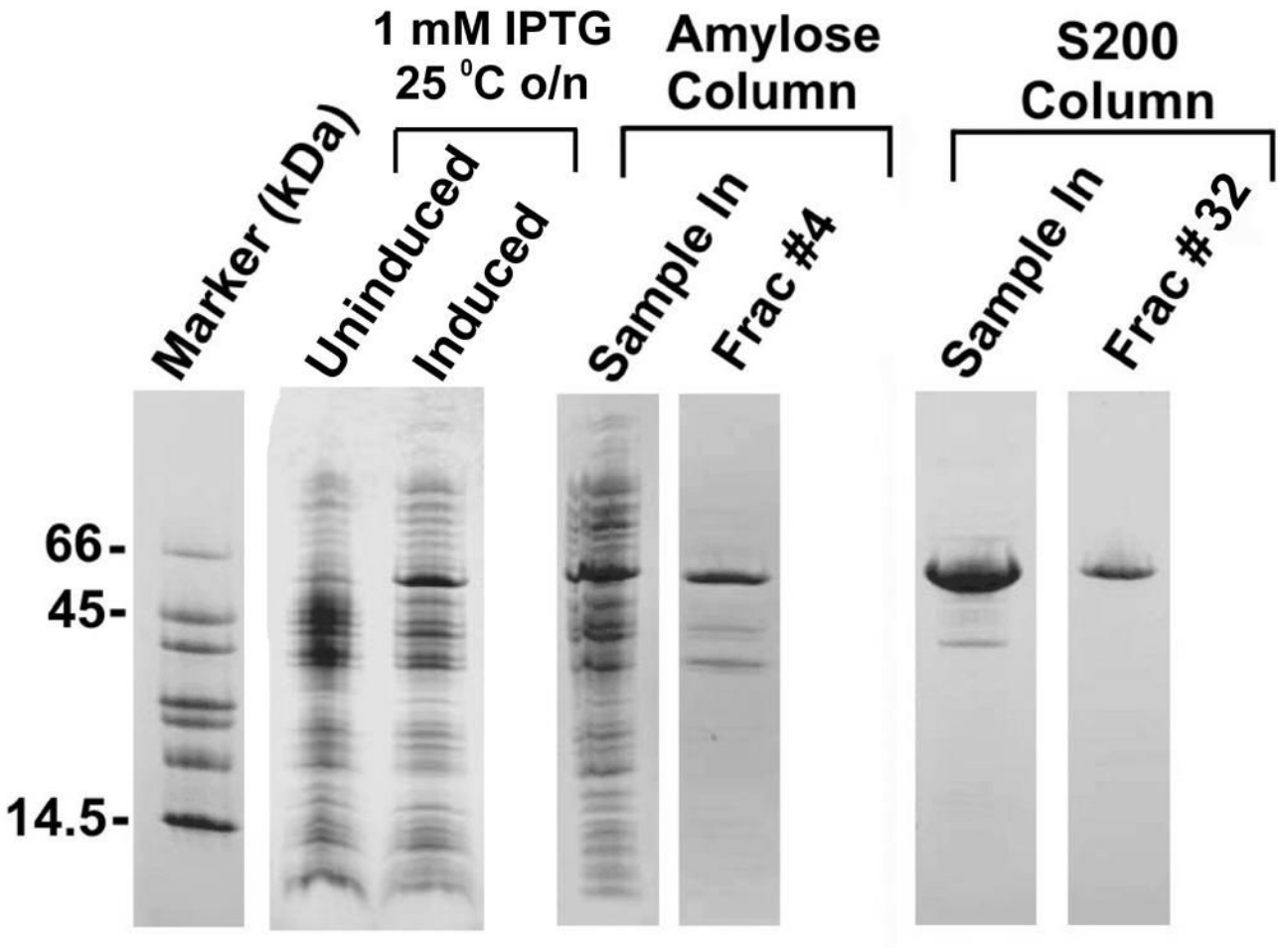


Figure 5.
Purification of MBP-hRCP. SDS-PAGE analysis showing the best purification scheme for MBP-hRCP fusion protein from soluble BL21(DE3) cell extract. An affinity column (amylose) was followed by a size exclusion step. All gels (8–25% Phast gels) were run under identical conditions. Only fractions containing the peak are shown. (Sample In: Sample applied on the given column.)

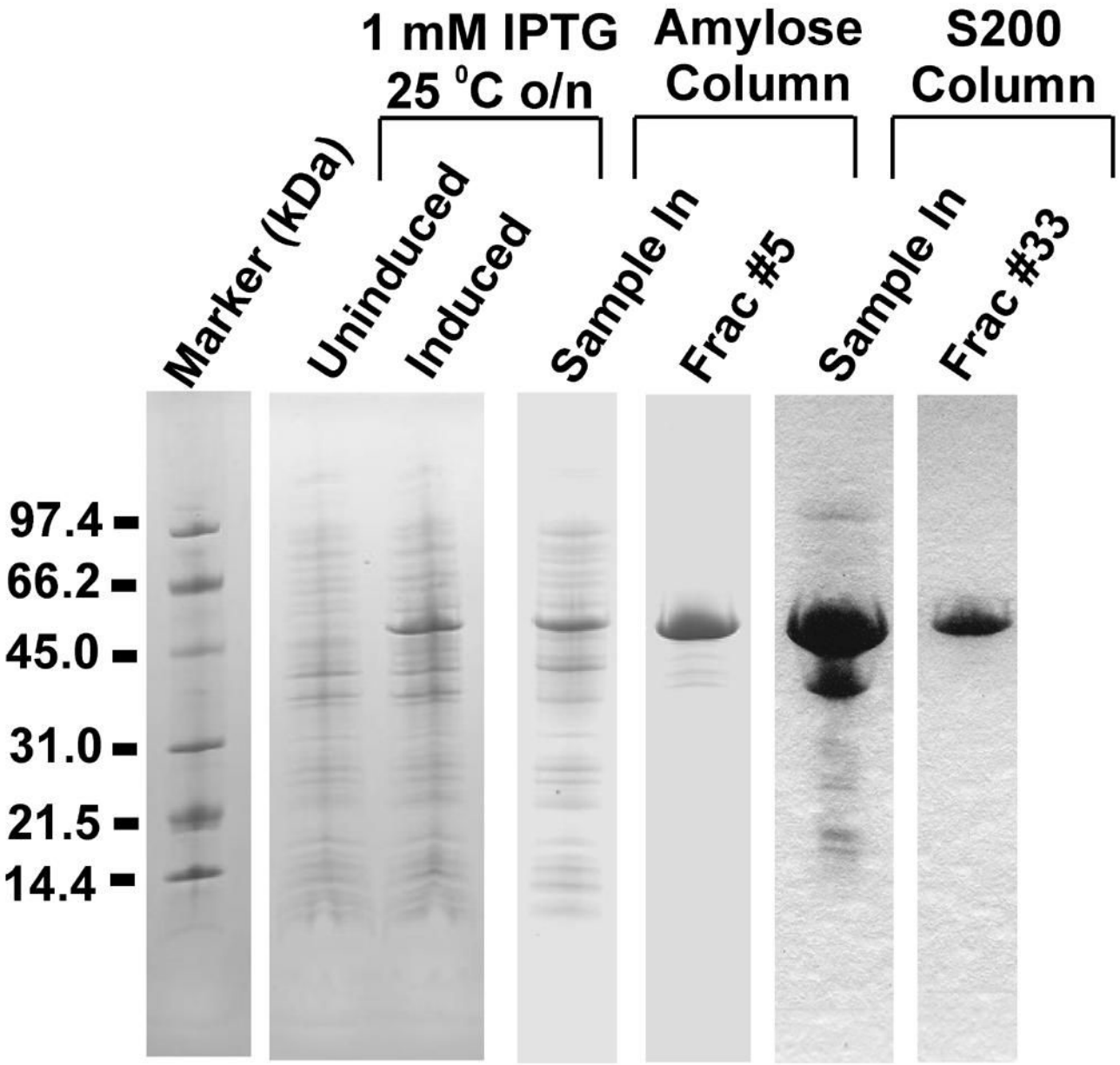


Figure 6.
Purification of MBP-shRCP. SDS-PAGE analysis showing the purification scheme for MBP-hRCP fusion protein with a short linker. An affinity column (amylose) was followed by a size exclusion step. All gels (8–25% Phast gels) were run under identical conditions. Only fractions containing the peak are shown. (Sample In: Sample applied on the given column.)

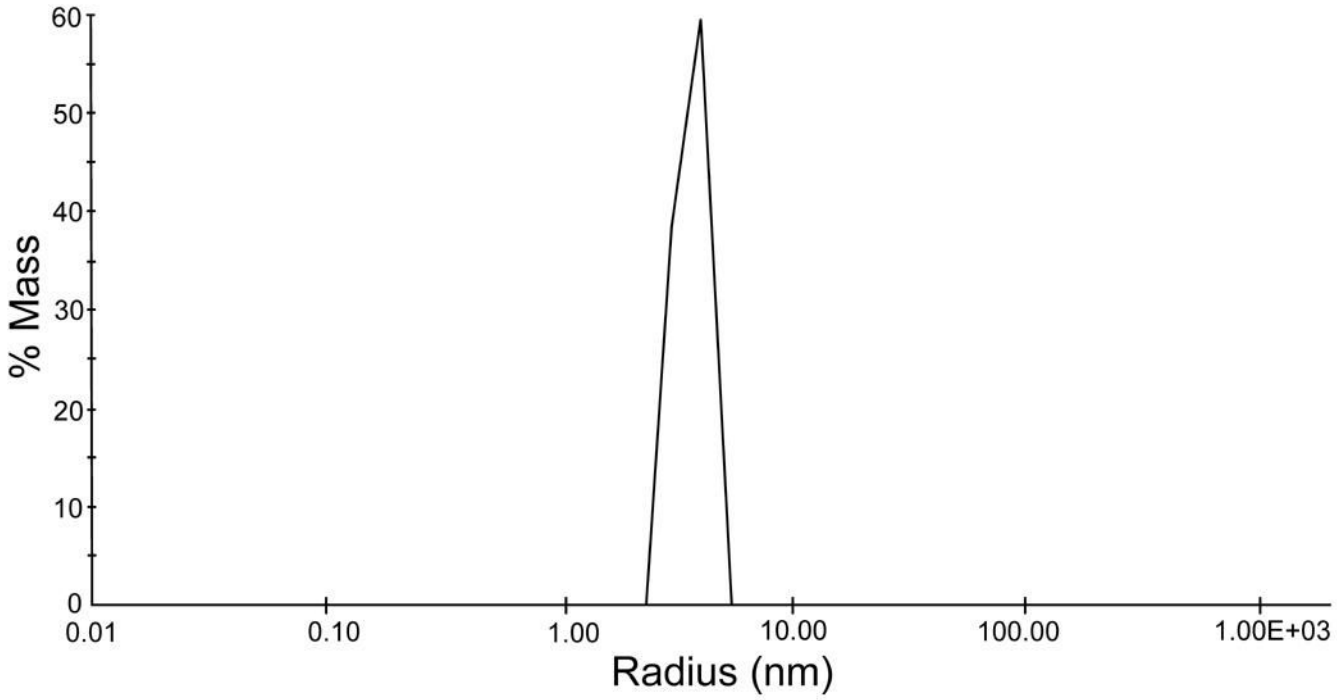
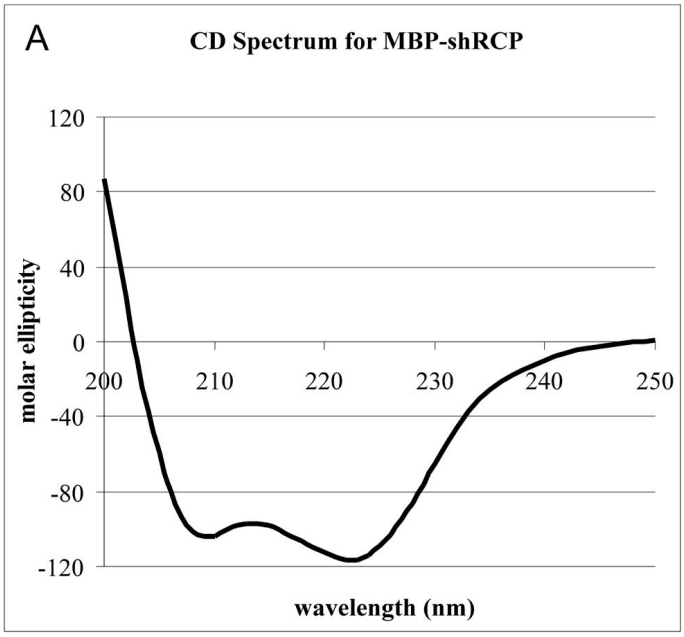


Figure 7.
Hydrodynamic radius distribution of MBP-shRCP derived from dynamic light scattering.



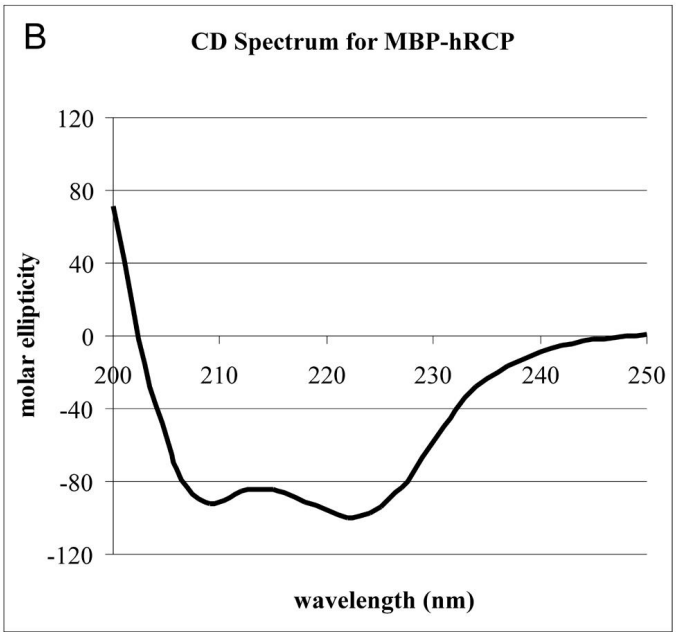


Figure 8.
Circular Dichroism Spectra of (A) MBP-hRCP, and (B) MBP-shRCP.

Table 1

Summary of histidine tagged hRCP purification.

	Total Protein (mg)	Purity (%)**	Overall Yield (%)***
Crude Extract*	210.0	24	100
Metal Affinity Column	37.0	37	18
MonoQ Column	14.7	47	7
S-200 Column	4.0	98	2

From 15 g of wet cell paste.

* Protein amount in soluble fraction after Ammonium sulphate and Polymin P clarification.

** Percent purity determined by gel densitometry.

*** Total protein at each step / total protein in crude extract.

Table 2

Summary of Maltose Binding Protein tagged hRCP (MBP-hRCP) purification.

	Total Protein (mg)	Purity (%)**	Overall Yield (%)***
Crude Extract*	200	16	100
Amylose Column	20	92	10
S-200 Column	6	99	3

From 3 g of wet cell paste.

* Protein amount in soluble fraction after Ammonium sulphate and Polymin P clarification.

** Percent purity determined by gel densitometry.

*** Total protein at each step / total protein in crude extract.

Table 3

Summary of Maltose Binding Protein tagged short linker hRCP (MBP-shRCP) purification.

	Total Protein (mg)	Purity (%)**	Overall Yield (%)***
Crude Extract*	500	16	100
Amylose Column	50	43	10
S-200 Column	4	99	1

From 5 g of wet cell paste.

* Protein amount in the soluble fraction after Ammonium sulphate and Polymin P clarification.

** Percent purity determined by gel densitometry.

*** Total protein at each step / total protein in crude extract.