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In vitro refolding of recombinant human free secretory component using equilibrium gradient dialysis

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Abstract

Human secretory component (SC) is associated with secretory immunoglobulins (IgA and IgM) and serves to protect the immunoglobulin in the harsh mucosal environment. SC is derived from the polymeric immunoglobulin receptor (pIgR) which transports polymeric immunoglobulins across epithelial cells into secretions. In this present study, we describe the first cloning, expression, in vitro refolding and purification of a free form of human secretory component (rSC) containing the five functional ligand binding domains using *Escherichia coli* BL21 (DE3). Free rSC was refolded from inclusion bodies by equilibrium dialysis after purification by nickel affinity chromatography under denaturing conditions. Refolded rSC was purified by gel filtration chromatography. Surface plasmon resonance and dot blot association analysis have shown that purified rSC binds IgM with a physiological equilibrium dissociation constant (K_D) of 4.6×10^{-8} M and shares structural similarity to native SC. This provides an important step in the elucidation of the structure of this immunologically important receptor.

Keywords: Human; Fc receptors; Immunoglobulins; Polymeric immunoglobulin receptor; Secretory component

Secretory immunoglobulins such as polymeric IgA, dimeric IgA (dIgA), and pentameric IgM (pIgM) are the first line of defense against microbial infection at the mucosal surface [1]. A polypeptide termed secretory component (SC, ~86,000 Da) was first described by Tomasi et al. [2] as a polypeptide bound to secretory immunoglobulins. Lindh [3] attributed a protective function to the molecule, protecting the polymeric immunoglobulin against proteolytic cleavage in the mucosal environment. Mostov and coworkers [4–6] showed that secretory component (SC) was derived from a transmembrane receptor, termed the polymeric immunoglobulin receptor (pIgR),[1] expressed on the basolateral surfaces (where binding to dIgA or pIgM occurs) of epithelial cells and effects the transfer (transcytosis) of polymeric immunoglobulins across the cell to the apical surface, at which point a cleavage event occurs (at Arginine-585, shown by Hughes et al. [7]) and the complex is released as a secretory immunoglobulin. The pIgR molecule is composed of three regions: the extracellular ligand binding region; the transmembrane domain and the cytoplasmic tail. The transmembrane domain and cytoplasmic region contain highly conserved amino acid residues attributed to signal sequences [8–10]. The extracellular ligand binding region is composed of five homologous domains (denoted by roman numerals I through V) with similarity to immunoglobulin variable regions [6] containing 20 cysteine residues that form 10 disulfides bridges [11]. Of these domains, domain I was shown to be responsible for the initial non-covalent interaction with both IgA and IgM [12–15]. Little is known of the function of domains II, III, and IV. Domain V is involved in a covalent disulfide

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[1] *Abbreviations used:* pIgR, polymeric immunoglobulin receptor; CbpA, choline binding protein A; BSA, bovine serum albumin; EDC, 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide; NHS, *N*-hydroxysuccinimide; BCA, bincinchoninic acid.

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linkage with IgA [11,16]. No detectable function has been attributed to domain V in IgM binding.

Typically, mucosal secretions also contain large amounts of free SC. Of special note is the fact that in fetal development pIgR and SC production precedes IgA production by months [17]. This alludes to other unknown functions of pIgR/SC. Zhang et al. [18] showed the binding of pneumococcal choline binding protein A (CbpA) (via domains III and IV; [19]) and translocation of *Streptococcus pneumoniae* by pIgR across nasopharyngeal epithelial cells allowing for microbial infection. In healthy individuals the free SC may maintain a homeostatic relationship preventing pneumococcal invasion. The ability of free SC to bind enterotoxigenic *Escherichia coli* was demonstrated by de Oliveira et al. [20]. Studies have shown that human eosinophils express receptors for free SC [21] and stimulate effector functions [22]. It is clear that other roles for free SC exist. The potential of the molecule as a drug delivery vehicle to epithelial cells and the mucosa was shown by Ferkol et al. [23].

Native SC molecules are typically purified from breast milk using IgM affinity chromatography [24], a time consuming method. The production of recombinant murine SC has been described in HeLa cells [25]. Attempts to produce secretory component in non-mammalian cells, like plants, are typically limited to coexpression with IgA and therefore formation of secretory antibodies [26] or expression in insect cells [27]. All methods described are costly and require highly specialized equipment. Bakos et al. [28] produced a functional domain I molecule in the bacterial expression system *E. coli* BL21 (DE3); based on this Hamburger et al. [29] elucidated the crystal structure of domain I.

In this paper, we report the production and in vitro refolding of a 552 amino acid functional, human, free secretory component (rSC) in the bacterial expression system *E. coli* BL21 (DE3). This may lead to further studies of the interaction with immunoglobulins and elucidation of the complete structure of free SC.

Materials and methods

Reagents

The RNeasy Minikit was from QIAgen GmbH (Hilden, Germany); Titan One Tube RT-PCR kit, isopropyl-β-D-thiogalactopyranoside (IPTG), lysozyme, PVDF membrane, and bovine serum albumin (BSA) from Roche (Mannheim, Germany); pCRScript SK (+) Cloning kit from Stratagene (Cedar Creek, Texas, USA); pET22b (+) and *E. coli* BL21 (DE3) from Novagen (Madison, Wisconsin); *E. coli* DH5 (α) from Invitrogen GmbH (Karlsruhe, Germany). The HIS-Select nickel affinity resin, bacterial protease inhibitor cocktail, Tween 20, Micro-Biotinylation Kit, polyclonal anti-human secretory component, IgM, extravidin-peroxidase conjugate, SigmaFast 3, 3-diaminobenzidine were from Sigma–Aldrich (St. Louis, Missouri, USA). The Superdex 75 (26/60) FPLC column was from Amersham–Pharmacia Biosciences AB (Uppsala, Sweden); Amicon Ultra-15 5k centrifugal filters from Millipore (Cork, Ireland); CM5 sensor chip, HBS-EP (10 mM Hepes, pH 7.4, 150 mM NaCl, 3.4 mM EDTA, and 0.005% Surfactant P20), ethanolamine, 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) from Biacore AB (Uppsala, Sweden). *Nde*I and *Xho*I were from New England Biolabs (Massachusetts, USA). All reagents were of the highest analytical grade available.

Cloning and expression of unfolded recombinant free SC

Human pIgR was amplified from mRNA isolated from HT-29 cells grown in monolayer using QIAgen RNeasy Minikit. RT-PCR was performed with the Titan One Tube RT-PCR kit with gene specific primers for the pIgR ectodomain (5′ primer AAA AAA ACA TAT GAG TCC CAT ATT TGG; 3′ primer AAG AAT TCA GGA GCT TCC ACC TTG). PCR product was cloned and screened for in *E. coli* DH5 (α) using the pCRScript SK (+) Cloning kit. Free SC was amplified by PCR using domain specific primers based on the 5′ border of domain I (5′ AAA AAA ACA TAT GAG TCC CAT ATT TGG 3′) and 3′ border of domain V (5′ AAA ACT CGA GAA CTG CCA CAT AG 3′) and amplified PCR fragments were blunt ligated into pCR Script SK (+). Plasmids were isolated from positive clones and restricted with *Nde*I and *Xho*I; fragments were isolated and ligated into pET22b (+) prior to transformation into *E. coli* BL21 (DE3). Positive clones, confirmed by DNA sequencing, were screened for protein expression by IPTG induction of 50 ml cultures. Production of recombinant protein and formation of inclusion bodies were determined by 15% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) [30].

Preparative expressions of inclusion bodies were performed after determination that the proteins produced were not in the soluble fraction (data not shown). Four hour preparative expressions (4 L) were performed at 37 °C using log phase cells induced with 1 mM IPTG. The cells were harvested by centrifugation (5000*g* for 5 min) and resuspended in sonication buffer (50 mM Tris–HCl, 0.3 M NaCl, 1 mM EDTA, and 0.2% NaN_3). Inclusion bodies were isolated by addition of lysozyme (2 mg/ml) to the cell paste and incubation at room temperature for 1 h. The cell paste was sonicated [HD2200 Sonopuls (Bandelin Electronics GmbH, Berlin, Germany) fitted with a UW2200 probe] at 50% duty cycle, 40% power for three bursts of 6 min and then centrifuged for 40 min at ~42,000*g*. The cell pellet was homogenised and washed with inclusion body wash buffer (50 mM Tris–HCl, 0.3 M NaCl, 1 mM EDTA, 0.2% (w/v) NaN_3, and 0.5% (w/v) *N*,*N*-dimethyldodecylamine *N*-oxide) to remove soluble *E. coli* proteins. The final pellet was homogenised in sonication buffer and stored at 4 °C in the presence of bacterial protease inhibitor cocktail. Protein concentration was assayed by using both the bincinchoninic acid (BCA) [31] and the Bradford assay [32].