Biochimica et Biophysica Acta, 1152 (1993) 1–8

BBAMEM 76057

A 56 kDa binding protein for *Escherichia coli* heat-stable enterotoxin isolated from the cytoskeleton of rat intestinal membranes does not possess guanylate cyclase activity

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(Received 22 February 1993)

Key words: Enterotoxin; Intestinal membrane; Cytoskeleton; Guanylate cyclase activity; Receptor binding; (Rat); (*E. coli*)

Proteins binding *Escherichia coli* heat-stable enterotoxin were isolated from the cytoskeleton of intestinal membranes using an affinity matrix of biotinylated ST immobilized on monomeric avidin-agarose. ST binding proteins were purified 343-fold using this affinity technique, with 7% of the initial binding activity recovered in these preparations. ST binding proteins isolated by affinity chromatography possessed a native and subunit molecular mass of 56 kDa. These preparations exhibited both high- and low-affinity binding sites for ST. Guanylate cyclase in extracts of the intestinal membrane cytoskeleton was completely recovered in fractions which did not associate with the affinity matrix. In addition, ST binding proteins isolated by affinity chromatography were devoid of guanylate cyclase activity. These data, taken together with those obtained previously with crude and partially purified receptors, suggest that ST binds to different proteins in intestinal membranes, some of which do not possess guanylate cyclase activity.

Introduction

Escherichia coli heat-stable enterotoxin (ST) is a major etiologic agent in the production of endemic diarrhea in developing countries and travelers diarrhea [1,2]. ST induces intestinal secretion by binding to specific protein receptors on brush-border membranes of mucosal cells [3–5]. Interaction of ST with receptors activates particulate guanylate cyclase, resulting in the intracellular accumulation of guanosine 3′,5′-cyclic monophosphate (cyclic GMP) which directly mediates alterations of intestinal fluid and electrolyte secretion [6–9].

Previous studies suggest that ST receptors exhibit significant heterogeneity of subunit structure, ligand affinity, and subcellular localization [5,10–18]. Recently, a protein was cloned from intestinal cell cDNA libraries which possesses both ST binding and guanylate cyclase activities on a single transmembrane protein [19–22]. Crosslinking of this protein, expressed in mammalian cells, to labeled ST resulted in the identification of toxin binding proteins of high and low molecular weights, similar to those identified in native intestinal cells [11–15,17,18,21]. These data suggest that heterogeneous ST receptors may be derived by post-translational modification from a single precursor which belongs to the family of particulate guanylate cyclases possessing ligand binding activity [19–23]. Alternatively, these data could be consistent with the presence of multiple populations of different ST receptors only some of which are co-localized with guanylate cyclase on the same transmembrane protein.

ST binding proteins have been purified from the lipid bilayer of intestinal membranes using an affinity column of biotinylated toxin immobilized on monomeric avidin-agarose [24]. These proteins exhibited a subunit molecular mass of 74 kDa, bound ST in a concentration-dependent fashion, and were devoid of guanylate cyclase activity. However, technical difficulties associated with these preparations did not permit the resolution of whether purified ST receptors possessed guanylate cyclase activity [24]. Previous studies demonstrated that ST receptors associated with the cytoskeleton of intestinal membranes are preferentially coupled to activation of guanylate cyclase [15,18]. The present study was directed at establishing the chromatographic be-

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havior on the ST affinity matrix of toxin binding proteins and particulate guanylate cyclase extracted from the cytoskeleton of intestinal membranes, to determine whether these activities are localized on the same or different proteins.

Materials and Methods

Materials

Native ST was purified from *Escherichia coli* strain 431 as described previously [4]. NHS-LC-biotin, disuccinimidyl suberate (DSS) and monomeric avidin immobilized on 4% agarose were obtained from Pierce (Rockford, IL). Centriprep-30 and Centricon-30 were obtained from Amicon (Beverly, MA). Biotin and polyoxyethylene-9-lauryl ether (Polidocanol) were obtained from Sigma (St. Louis, MO). Gel filtration HPLC was conducted on an Ultraspherogel SEC 3000 column from Beckman Instruments (San Ramon, CA). Hydroxyapatite HPLC was conducted on a column from Bio-Rad (Richmond, CA). Sep-Pak columns were obtained from Waters (Milford, MA). All other reagents were of the highest analytical reagent grade and obtained as described previously [15,18].

Membrane preparation and solubilization

Rat intestinal membranes were prepared as described previously [14–16,18]. Briefly, rats were killed, small intestine removed, and washed in ice-cold buffer containing 0.9% NaCl followed by 50 mM Tris-HCl (pH 7.6), 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF; TED buffer), and 0.25 M sucrose. Intestinal mucosa was removed and homogenized in TED buffer containing 0.25 M sucrose. The homogenate was centrifuged at $100\,000 \times g$ for 60 min and pellets sequentially washed in TED containing 0.5 M KCl, TED containing 0.125 M sucrose, and TED. The final pellet was suspended in TED and stored as aliquots of 8–14 mg of protein/ml protein at −70°C.

Solubilization of ST binding and guanylate cyclase activities from the cytoskeletal matrix was performed as described previously [15,18]. Briefly, membranes (4 mg/ml protein) were incubated at 4°C for 1.5 h in buffer containing 50 mM Tris (pH 7.6), 0.2% Polidocanol (v/v), 1 mM DTT, 0.1 mM PMSF and 20% glycerol (solubilization buffer) and centrifuged at $100\,000 \times g$ for 60 min. The resulting pellet was suspended in solubilization buffer containing 0.4 M KCl, homogenized by 10 vigorous strokes by hand in a Teflon-on-glass homogenizer followed by vortexing at full speed for 1 min, and centrifugation at $100\,000 \times g$ for 60 min [25]. Supernatants contained guanylate cyclase and ST binding activities selectively extracted from the cytoskeleton of intestinal membranes.

Receptor binding assays

ST binding was quantified as described previously [14–16,18]. Briefly, samples were incubated with ^{125}I-ST (1000 Ci/mmol) in 50 mM Tris (pH 7.6) containing 1 mM EDTA, 133 mM KCl, 0.1% bacitracin, and 0.67 mM cystamine (binding buffer) for 2 h at 37°C to achieve equilibrium binding. In assays of crude receptors, 10^{-9} M ^{125}I-ST was employed while assays of affinity-purified toxin binding proteins utilized 10^{-8} M ^{125}I-ST. In experiments examining the concentration-dependence of toxin binding, affinity-purified preparations chromatographed on hydroxyapatite to remove free biotinylated ST were employed. In these experiments, the concentration of ^{125}I-ST was varied from 10^{-12} to 10^{-8} M. After incubation, reactions were filtered under vacuum on glass fiber filters (Whatman GF/B) to separate free from bound ^{125}I-ST. Radioactivity retained on filters was quantified using a Packard gamma counter. Specific binding was determined by subtracting from total binding non-specific binding estimated in parallel incubations in the presence of 0.1 μM unlabeled ST. All curves were fitted and binding constants determined using the program CIGALE on an Apple Macintosh SE 30 [14–16,18]. CIGALE was written by M. Bordes (Institut de Pharmacologie Cellulaire et Moleculaire, CNRS, 660 Rte des Lucioles, 06560 Valbonne/Sophia Antipolis, France). Using this program, ligand binding was analyzed as previously described [14,16,18,24,26].

Guanylate cyclase assay

Guanylate cyclase was assayed as described previously [14–16,18]. Reaction mixtures contained membranes, solubilized preparations or purified ST receptors (10–40 μl), 50 mM Tris-HCl (pH 7.6), 10 mM theophylline, 80 mM KCl, a GTP-regenerating system (15 mM creatine phosphate and 2.7 Units of creatine phosphokinase) and Mn^{2+}-GTP (4:1 mM), unless otherwise stated. Reactions were initiated by the addition of membranes, incubated at 37°C for 5 min, and terminated by addition of ice-cold 50 mM sodium acetate (pH 4.0), and boiling for 3 min. Samples were acetylated and the cyclic GMP produced was quantified by radioimmunoassay.

Biotinylation of ST

Biotinylated ST was prepared as described previously [24]. Briefly, native ST, containing ^{125}I-ST as a tracer, was mixed for 48 h at room temperature with a 20-fold molar excess of NHS-LC-biotin in 50 mM carbonate buffer (pH 9.0). Unreacted reagents were separated from biotinylated ST using a Sep-Pak column. Monomeric avidin immobilized on 4% agarose was utilized as the affinity support for the biotinylated toxin.

ST affinity column chromatography

ST affinity column chromatography was performed as described previously with some modifications [24]. Biotinylated ST ($3 \cdot 10^{-5}$ M) was incubated with monomeric avidin-agarose for 1 h at room temperature. Excess biotinylated ST was washed off the column using solubilization buffer and the final concentration of biotinylated ST immobilized on monomeric avidin was about 10^{-5} M. Extracts of intestinal membrane cytoskeleton were cycled over the column three times at a flow rate not exceeding 10 ml/h. The column was washed with 20 volumes of solubilization buffer and biotinylated ST bound to receptors eluted using solubilization buffer containing 2 mM biotin and 0.02% Polidocanol. Elution was followed by monitoring the amount of biotinylated ^{125}I-ST in eluant fractions. Eluants containing biotinylated ^{125}I-ST were pooled, concentrated using a Centriprep-30 and utilized for further analyses.

HPLC chromatography

ST receptors isolated by affinity chromatography were separated from free biotinylated ^{125}I-ST by HPLC on a hydroxyapatite column previously equilibrated with 30 mM sodium phosphate (pH 7.2), 1 mM DTT, 0.1 mM PMSF, 0.02% Polidocanol, and 0.05% NaN_3 (phosphate buffer). ST binding activity was eluted using phosphate buffer which was 300 mM in sodium phosphate. Also, affinity-purified ST binding proteins were subjected to HPLC gel filtration chromatography on a Ultraspherogel SEC 3000 column equilibrated with 100 mM sodium phosphate, 1 mM DTT, 0.1 mM PMSF, 0.02% Polidocanol, and 0.05% NaN_3. ST binding proteins eluted from the hydroxyapatite column were incubated in binding assays for 2 h in the presence of 10^{-8} M ^{125}I-ST (1000 Ci/mmol) with and without 0.1 μM ST and then chromatographed on the gel filtration column (total chromatography time 26 min). Fractions of 1 ml were collected and screened for bound ^{125}I-ST, and specific binding in each fraction calculated by subtracting from total binding that obtained in a parallel chromatographic run in the presence of excess unlabeled toxin. Previous studies demonstrated that ST dissociates slowly from receptors with a dissociation rate constant at 37°C of about $1.5 \cdot 10^{-2}$ min^{-1} [14]. Therefore, negligible dissociation of ligand and receptor occurs during HPLC gel filtration chromatography at room temperature. Indeed, HPLC gel filtration chromatography of samples bound to radiolabeled toxin has been employed previously to analyze the physical characteristics of crude ST receptors [5].

Receptor crosslinking

Radiolabeled ST was crosslinked to purified receptors as described previously [5,11,15,18,24]. Briefly, purified receptors (0.5 to 1.0 ml) were incubated for 2 h at 37°C in binding buffer containing 10^{-8} M ^{125}I-ST (1000 Ci/mmol). Parallel incubations contained 1000-fold molar excess of unlabeled ST to determine the specificity of crosslinking. DSS (100 mM) in DMSO was added to a final concentration of 1 mM, reactions incubated for 15 min at room temperature, and terminated by the addition of buffer containing 0.0625 M Tris (pH 6.8), 10% glycerol, 3% SDS, 0.004% Bromophenyl blue, and 5% β-mercaptoethanol and immersion in boiling water for 5 min. Crosslinked samples were analyzed by gradient SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and proteins visualized using a sensitive silver stain. Labeled proteins were identified by autoradiography using Kodak XARS film.

Miscellaneous

ST was iodinated to a final specific activity of 1000 Ci/mmol as described previously [14]. Protein was estimated using a modification of the Lowry technique [27]. All assays were performed in duplicate or triplicate and results are representative of at least three experiments.

Results

Solubilization of ST receptors and particulate guanylate cyclase

ST receptors and guanylate cyclase exhibited a heterogeneous distribution in rat intestinal membranes as described previously [10,15,18]. In the presence of solubilization buffer containing Polidocanol alone, 55% ± 11 and 38% ± 12 ($n = 4$) of the guanylate cyclase and ST binding activities, respectively, were solubilized from intestinal membranes. These activities presumably are localized in the lipid bilayer of intestinal mucosal membranes [10,15,18]. Extraction of residual membranes, previously exposed to detergent alone, with buffer containing Polidocanol and 0.4 M KCl resulted in the further solubilization of 36% ± 4 and 61% ± 15 ($n = 4$) of the guanylate cyclase and ST binding activities from the cytoskeleton of intestinal membranes [15,18,25,28]. ST (10^{-6} M) preferentially activated guanylate cyclase extracted from the cytoskeleton 8 ± 2-fold compared to activation of that enzyme in extracts of the lipid bilayer of 2 ± 1-fold ($n = 4$), as described previously [15,18].

Affinity chromatography of ST binding proteins

Detergent extracts of the cytoskeleton of intestinal membranes were subjected to ST affinity chromatography (Table I). About 50% to 70% of the ST binding activity in crude extracts was specifically retained by the affinity matrix, as observed previously with receptors extracted from the lipid bilayer [24]. Elution of the affinity resin with 2 mM free biotin specifically released biotinylated ST liganded to toxin binding pro-

TABLE I

ST receptor purification on biotinylated ST-monomeric avidin-agarose

Fraction	Total protein (mg)	Total activity (pmol) [a]	Specific activity (pmol/mg protein)	% recovery	Fold purification
Membrane [b]	1700 [f]	200	0.118	100	1
Extract [c]	144	76	0.53	38	4.5
Effluent [d]	139	37.2	0.31	18.6	2.6
Eluant [e]	0.35	14.2	40.57	7.1	343.8

[a] ST binding was measured in different fractions using various concentrations of ST as described in Materials and Methods. In order to compare ST binding in each fraction, ligand binding is expressed as maximum binding (B_{max}), calculated according to the standard equation [14] $B_{max} = [(\text{observed binding})(\text{ligand concentration} + K_d)]/(\text{ligand concentration})]$.

[b] Intestinal membranes were prepared as described in Materials and Methods.

[c] ST receptors were solubilized from the cytoskeleton of intestinal membranes as described in Materials and Methods.

[d] ST binding activity was quantified in fractions flowing through the affinity column (Effluent).

[e] Eluants were obtained with buffer containing 2 mM free biotin as described in Materials and Methods.

[f] Data presented are from a single preparation of 600 mg of rat intestinal mucosa protein. These data are representative of at least three experiments.

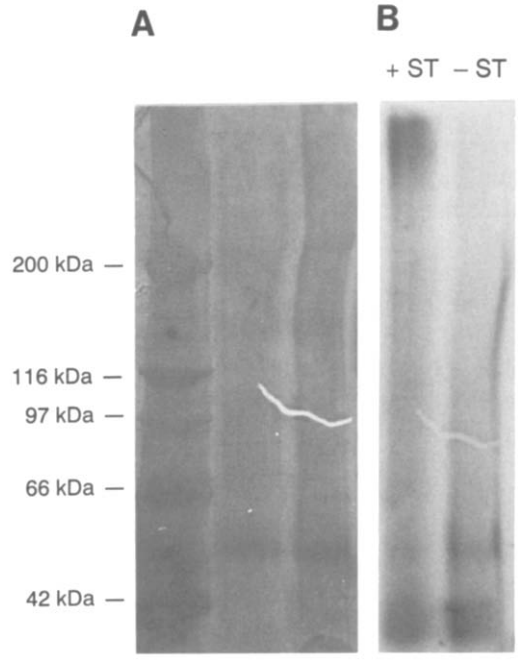

Fig. 1. SDS-PAGE analysis of ST receptors purified by ligand-affinity chromatography. Biotin eluants (1 ml) of the toxin affinity column possessing ST binding activity were crosslinked to ^{125}I-ST (10^{-8} M; 1000 Ci/mmol) in the absence (−ST) and presence (+ST) of 0.1 μM ST and concentrated 10-fold in a Centriprep 30 as described in Materials and Methods. Crosslinked receptors (50 μl of the concentrated sample) were analyzed by SDS-PAGE, silver staining, and autoradiography as described. (A) Silver-stain of SDS-PAGE gel loaded with purified receptors crosslinked to ^{125}I-ST. (B) Autoradiogram of (A). Molecular mass standards are presented in the right lane.

teins. Using this technique, ST binding activity was purified 343-fold, with 7% of the total toxin binding activity in intestinal membranes recovered in affinity-purified preparations. Analysis of these preparations by SDS-PAGE demonstrated a major protein band of 56 kDa (Fig. 1A). Crosslinking of these preparations demonstrated that the 56 kDa protein specifically bound ^{125}I-ST (Fig. 1B).

Binding characteristics of affinity-purified ST binding proteins

Previous studies demonstrated the presence of two different ST binding affinities associated with the cytoskeleton of intestinal membranes [14–16,18] (Fig. 2A). Similarly, in the present studies, Scatchard analyses of equilibrium binding data were curvilinear, suggesting the presence of high-affinity, low-capacity binding sites and low-affinity, high-capacity binding sites for ST in preparations obtained by affinity chromatography (Fig. 2B). The affinities for these different sites compared closely with those obtained with crude extracts from the intestinal membrane cytoskeleton (Table II). Bind-

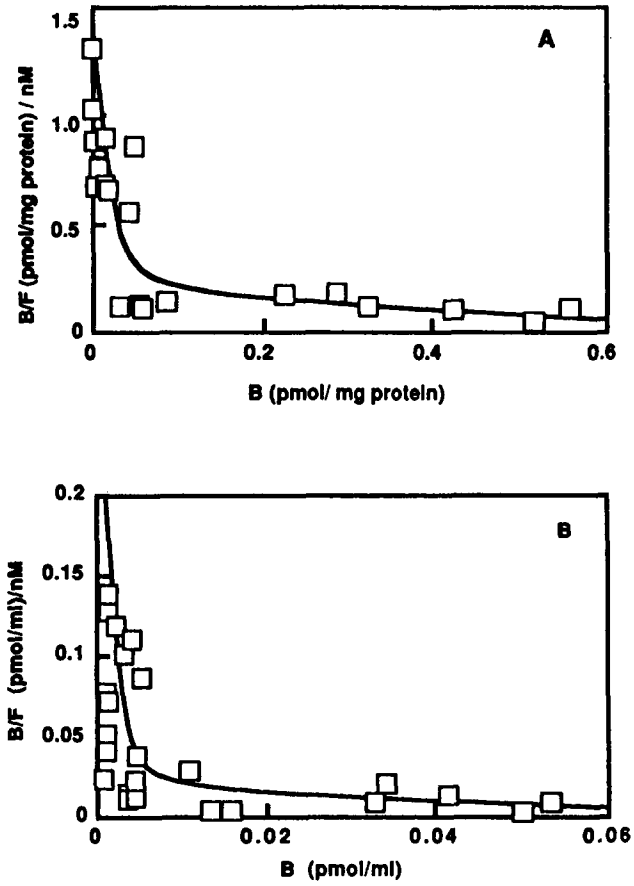

Fig. 2. Scatchard analyses of ST receptors extracted from the cytoskeleton of intestinal membranes (A) and after sequential ligand-affinity and hydroxyapatite chromatography (B). Equilibrium binding of receptors to ^{125}I-ST and the analyses of these data were performed as described in Materials and Methods. Scatchard analyses represent data pooled from three experiments.

TABLE II

Binding parameters of ST receptors purified by affinity chromatography [a]

Fraction	K_{dH} [b] (nM)	B_{maxH} (fmol/mg protein) (nM)	K_{dL}	B_{maxL} (pmol/mg protein)
Extract [c]	0.0325 ± 0.015 [d]	35 ± 19	2.57 ± 1.76	2.44 ± 1.69
Purified	0.0182 ± 0.008	n.d. [e]	3.06 ± 1.30	n.d.

[a] Dissociation constants and the maximum number of binding sites were determined for high- and low-affinity ST binding sites by analysis of Scatchard plots using the curve-fitting program CIGALE, as described in Materials and Methods and previously [14–16,18].

[b] K_{dH} and K_{dL} are the dissociation constants for the high- and low-affinity binding sites, respectively. B_{maxH} and B_{maxL} are the maximum number of binding sites for the high- and low-affinity sites, respectively.

[c] Extracts and affinity-purified receptors from the cytoskeleton of intestinal membranes were prepared as described in Materials and Methods. Purified receptors were subjected to hydroxyapatite chromatography by HPLC to remove free biotinylated ST, as described in Materials and Methods.

[d] Results are the mean of three determinations ± S.D.

[e] n.d., not determined. Concentrations of protein in samples of purified receptors obtained by hydroxyapatite chromatography, and required for the determination of maximum number of binding sites, were below the limit of detectability.

ing characteristics were determined using fractions chromatographed on hydroxyapatite, to remove free biotinylated ST which would interfere in equilibrium binding experiments. Protein concentrations of fractions from the hydroxyapatite column were below the limit of detectability. Thus, the maximum number of ST binding sites (B_{max}) in preparations obtained by affinity chromatography could not be determined. However, the ratio of high- and low-affinity sites in affinity-preparations, 4:100, agrees closely with that observed in intestinal membranes [15,18].

Gel filtration of affinity-purified ST binding proteins

Eluants of the hydroxyapatite column containing ST binding activity were subjected to gel filtration by HPLC (Fig. 3). Partially purified ST binding proteins migrated

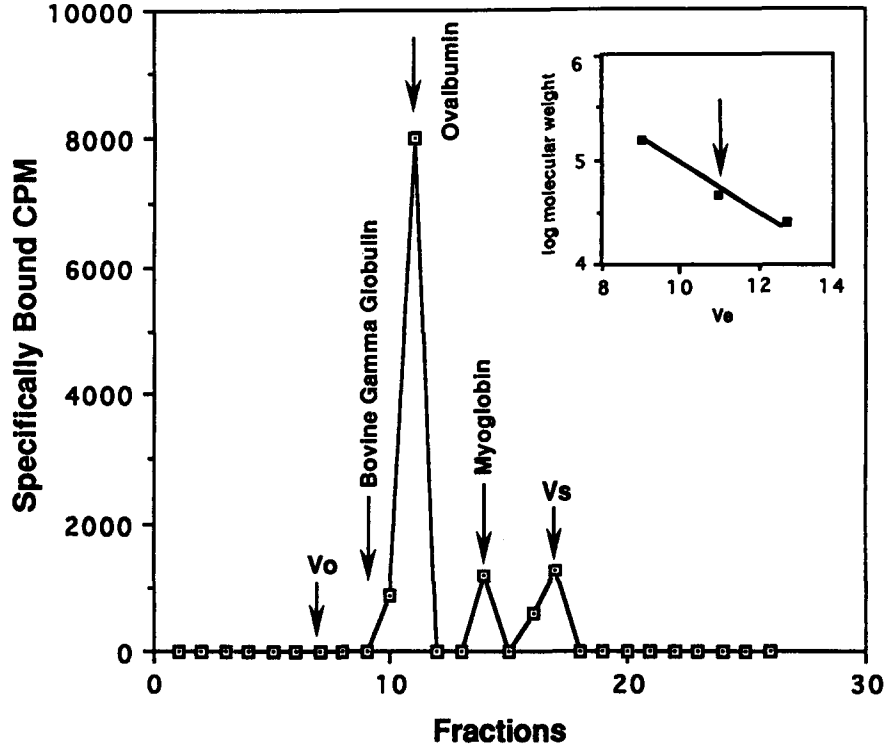

Fig. 3. Gel filtration chromatography of affinity-purified ST receptors. 15 ml (180 μg) of a purified preparation of ST receptors were concentrated to 1 ml and 500 μl were incubated with ^{125}I-ST (10^{-8} M) in the presence or absence of 0.1 μM unlabeled toxin and subjected to chromatography on an Ultraspherogel SEC 3000 column by HPLC as described in Materials and Methods. V_o, void volume; V_s, salt volume. The inset demonstrates the relationship between the elution fraction (V_e) and the molecular mass of the following standards: bovine γ-globulin (158 kDa), ovalbumin (66 kDa), and myoglobin (44 kDa). The arrow indicates the elution fraction of the peak of specific ^{125}I-ST binding.

TABLE III

Guanylate cyclase activity chromatographed on biotinylated ST-monomeric avidin-agarose

Fraction	Total protein (mg)	Total activity (pmol/min)	Specific activity (pmol/min per mg protein)	% recovery
Extract [a]	19.6 [c]	1580	80.6	100.0
Effluent [b]	17.8	1660	93.3	105.1
Eluant	0.10	n.d. [d]	n.d.	n.d.

[a] Guanylate cyclase was solubilized from the cytoskeleton of intestinal membranes as described in Materials and Methods.
[b] Guanylate cyclase activity was quantified in fractions flowing through the affinity column (Effluent).
[c] Data presented are from a single preparation. These data are representative of at least three experiments.
[d] n.d., not detectable.

as a single symmetrical peak of binding activity of 56 kDa. Binding was not detected at other molecular masses.

Particulate guanylate cyclase activity

The behavior of guanylate cyclase in extracts of intestinal membrane cytoskeleton subjected to ST-affinity chromatography was examined (Table III). Guanylate cyclase activity was quantitatively recovered in fractions flowing unbound through the column, with $107 \pm 27\%$ ($n = 3$) of the total enzyme activity recovered in column effluents. Also, guanylate cyclase activity was undetectable in fractions containing partially purified ST binding activity using Mg^{2+} or Mn^{2+} as the cation cofactor or in the presence of ST or adenine nucleotides, agents which stimulate this enzyme in crude preparations [15,18,29,30]. These data suggest that ST binding activity partially purified from the cytoskeleton of rat intestinal membranes by affinity chromatography is contained on a protein which is separate from guanylate cyclase.

Discussion

In this report, ST binding proteins were partially purified 343-fold from the cytoskeleton of rat intestinal membranes using an affinity column of biotinylated toxin immobilized on monomeric avidin-agarose. These preparations contained one major ST binding protein which was a monomer of 56 kDa. This toxin binding subunit has been identified previously in intestinal membranes and extracts of the lipid bilayer and cytoskeleton from those membranes [5,11,18]. Presumably, this subunit is similar to the 53 kDa ST-binding protein identified using a specific photoaffinity probe derived from the toxin [17]. ST binding proteins obtained by affinity chromatography were devoid of guanylate cyclase activity supporting the suggestion that subpopulations of this enzyme and ST binding are localized on different proteins.

In the present studies, high- and low-affinity binding sites for ST were observed in preparations obtained by toxin affinity chromatography. Earlier studies suggested that these sites reflected the presence of different receptor populations in intestinal membranes [14,15,18]. Similarly, preparations obtained by affinity chromatography may contain high affinity receptors which are below the limit of detection of the cross-linking technique. Alternatively, the 56 kDa receptor may undergo post-translational modifications, such as proteolysis or alterations in phosphorylation, resulting in heterogeneous binding characteristics.

A novel family of structurally homologous transmembrane proteins has been described, possessing peptide ligand binding and guanylate cyclase activities [23]. A 140 kDa protein cloned from mammalian intestinal cell cDNA libraries and encoding ST binding and guanylate cyclase activities is the most recent addition to this family [19–23]. Expression of this protein in mammalian cells in vitro resulted in the appearance of ST binding proteins of different molecular weights that were similar to those described previously in studies of ST receptors in intestinal membranes [11–15,17,18,22]. These data suggest that ST receptors in native intestinal cells represent a single translation product possessing toxin binding and guanylate cyclase activities on the same transmembrane protein but which undergo post-translational processing, presumably proteolysis, to yield binding subunits of different molecular weights [22].

In contrast, data obtained with receptors from native intestinal membranes suggest that there are populations of ST binding proteins which do not possess guanylate cyclase activity. Thus, guanylate cyclase activity can be completely separated from ST binding in extracts of intestinal membranes [5,10,17]. Similarly, ST binding proteins partially purified from the lipid bilayer do not possess detectable guanylate cyclase activity [24]. However, these data are difficult to interpret since partially purified samples were exposed to bile salts, phosphate-containing buffers, and elevated temperatures which could irreversibly inhibit guanylate cyclase [24]. In the present studies, affinity chromatography was modified to avoid exposing preparations to conditions that would inhibit guanylate cyclase activity. These studies demonstrate that in cytoskeleton extracts, guanylate cyclase completely flowed through the ST affinity column and was undetectable in partially purified fractions.

These data suggest that some forms of guanylate cyclase and ST binding activities may be contained on different proteins in intestinal membranes, in addition to those activities recently cloned and co-localized on a single transmembrane protein. The relationship be-

tween proteins possessing ST binding alone and those possessing ligand binding and guanylatye cyclase activities remains unclear. Different ST binding proteins may reside in intestinal membranes, some of which may represent the receptor-cyclase species. This hypothesis is consistent with the heterogeneity of structure and function which has been demonstrated for crude and partially purified ST receptors previously [14,16,17,24]. Also, it is in agreement with the observation that ST binding proteins in intestinal membranes with core peptides of 53 kDa diverge in their glycosylation characteristics and migration patterns on two-dimensional polyacrylamide gels [17]. These data suggest that the ST binding proteins from which those core peptides were derived are different, supporting the hypothesis that multiple ST receptors are present in intestinal membranes [17].

There is precedence for multiple receptors for a peptide ligand, some of which are transmembrane proteins possessing guanylate cyclase activity. Indeed, atrial natriuretic peptide (ANP) receptors are heterogeneous in most cells, a minority possessing a molecular mass of about 130 kDa and containing both guanylate cyclase and ANP binding activities, while the majority are homodimers of 66 kDa subunits devoid of guanylate cyclase activity [23]. ST receptors may exhibit similar heterogeneity, with some receptors consisting of the cloned protein possessing toxin binding and guanylate cyclase activities while others are oligomers of 74 or 56 kDa subunits devoid of cyclase activity.

Alternatively, ST receptors may be synthesized as a monomer possessing enzyme and ligand-binding activities, but undergo post-translational processing by proteinases [19–23]. Proteolytic cleavage could yield the heterogeneity in subunit structure, function, and subcellular distribution observed in previous studies with crude receptors. In addition, proteolysis could result in the isolation of ST binding proteins of 56 and 74 kDa by affinity chromatography which are devoid of guanylate cyclase activity. Finally, post-translational processing could result in the separation of guanylate cyclase and ST receptor binding activities by various biochemical techniques. However, in the present studies, 100% of the guanylate cyclase extracted from the cytoskeleton was recovered in fractions flowing through the affinity matrix. If ST binding and guanylate cyclase activities were contained on a single protein which undergoes post-translational processing, some of the parent receptors possessing both activities should be specifically retained and eluted from this matrix. In addition, ST binding proteins resulting from proteolysis of a common parent receptor should exhibit homologous glycosylation characteristics. However, at least some of these subunits are divergent in their glycosylation characteristics, suggesting that they are not derived from a common precursor [17].

In conclusion, extracts of the cytoskeleton of intestinal membranes were subjected to ST affinity chromatography. ST binding proteins in these extracts quantitatively associated with the affinity resin and were partially purified by specific elution. In contrast, guanylate cyclase in these extracts of intestinal membranes was quantitatively recovered in fractions which did not associate with the affinity matrix. In addition, affinity-purified preparations lacked guanylate cyclase activity. These data, taken together with those obtained previously with crude and partially purified receptors, suggest that ST binds to different proteins in intestinal membranes, some of which do not possess guanylate cyclase activity.

Acknowledgments

This work was supported, in part, by grants from the W.W. Smith Charitable Trust and NSF. Scott A. Waldman was the recipient of a PMA Faculty Development Award in Pharmacology and Toxicology.

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