Purification and Characterization of Monomeric *Escherichia coli* Vitamin B$_{12}$ Receptor with High Affinity for Colicin E3*

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The *btuB* gene product from *Escherichia coli* is a 66.5-kDa integral outer membrane protein required for high-affinity uptake of cyanocobalamin and the translocation of E group colicins and colicin A. Efficient purification of overexpressed BtuB containing stoichiometric levels of bound lipopolysaccharide has been achieved through the extraction of the outer membrane with nonionic detergent followed by ion-exchange chromatography. Analysis of far UV circular dichroism spectra indicates a predominantly β-sheet secondary structure (76 ± 4%) with a low α-helical content (15 ± 3%), providing the first direct evidence for secondary structure models derived from sequence and hydropathy analysis. Characterization of the octylglucoside-solubilized receptor by sedimentation equilibrium and sedimentation velocity analysis reveals a monodisperse protein-detergent complex of approximately 89 kDa with a sedimentation coefficient of 4.7 S which, after correction for bound detergent, indicates that BtuB is purified as a monomer. BtuB binds vitamin B$_{12}$ with a stoichiometry of approximately 1:1, as observed by a shift in the sedimentation profile of the vitamin to the much faster velocity observed for the protein-detergent complex. The preincubation of colicin E3 with stoichiometric levels of BtuB protects susceptible strains from the lethal effects of the colicin and results in a complex with a sedimentation coefficient appropriate for a BtuB-detergent-colicin E3 complex, demonstrating that monomeric BtuB retains high affinity for this particular ligand after isolation.

The outer membrane of *Escherichia coli* and other Gram-negative bacteria has evolved to exclude harmful agents present in the surrounding environment while maintaining the ability to internalize required nutrients of low abundance. Although general barrier function is largely provided by the lipopolysaccharide comprising the outer leaflet of this asymmetric bilayer (1, 2), the complexities of nutrient uptake are addressed by a variety of integral membrane proteins that operate by passive diffusion, facilitated diffusion, or active transport (3, 4). Bacterial porins form trimeric, aqueous-filled pores that are apparently coupled to extensive loop regions that are extensive compared with those observed in porin structures. The recent spectroscopic analysis of FhuA (12, 13) has provided the first direct evidence for a predominantly β-sheet secondary structure for detergent-solubilized receptor retaining the ability to bind both bacteriophage T5 and ferriichrome. Insight into the function of FhuA (14) and FepA (15) has been obtained through electrophysiological characterization of deletion mutants that, in contrast to the closed state observed for the wild-type protein, form relatively large, permanently open, aqueous diffusion pores after the excision of surface-exposed loops thought to be critical for channel gating. Although the monomeric state observed for detergent-solubilized FhuA (13) contrasts with porin quaternary structure (6), the available data has prompted the concept of TonB-dependent receptors as gated porins comprised of large, aqueous pores buried within the outer membrane. These pores are apparently coupled to extensive loop regions that provide the additional determinants required for function as selective, energy-dependent solute transporters.

The *btuB* gene product from *E. coli* is a 66,412-dalton, TonB-dependent receptor essential for high-affinity uptake of cyanocobalamin that also serves as a receptor for the E group colicins, colicin A, and phage BF23 (16). Although the ligand binding properties of purified receptor have been described previously (17, 18), and insights into both ligand binding (19, 20) and the mechanism of cobalamin transport (21, 22) have resulted from the characterization of BtuB mutants incorporated into the outer membrane, the lack of a purification procedure with a high yield has precluded a more detailed understanding of receptor properties. The present work describes...
procedures for overexpression and purification of the *E. coli* vitamin B₁₂ receptor in quantities sufficient for characterization and crystallization trials and provides information on the secondary structure, aggregation state, and ligand binding properties of the purified, protein-detergent complex.

**EXPERIMENTAL PROCEDURES**

Reagents—Mega-10 (Anagrade) and octylglucoside (Sol grade) were obtained from Antracite, Inc. Trypsine and yeast extract were purchased from Difco, DEAE-Sepharose CL-6B and Sephadex G-15 were purchased from Sigma and Pharmacia, respectively, and low molecular weight protein standards were purchased from Bio-Rad. Vitamin B₁₂ and LPS¹ (E. coli serotype 055:B5 and EH100) were obtained from Sigma. Colicin E1 (23) and colicin E3 (24) were purified as described previously.

Overexpression of BtuB—The outer membrane protein-deficient *E. coli* strain TNE012 (K12 tetx *ompA* *ompB*) was used for overexpression from plasmid pJC3 (25). Cells grown at 37 °C to an OD₆₀₀=1.0 in RB medium (10 g of bactotryptone and 5 g of yeast extract per liter of 50 mM NaKPO₄, pH 7.5) were harvested and stored at −20 °C until further use.

Outer Membrane Isolation and Extraction—Frozen cell pellets from 4 liters of culture were resuspended to approximately 20 ml with 200 ml Tris, pH 7.5 (pH of all buffers was adjusted at room temperature). Lysed (30 min) pellets were washed in 200 ml Tris and added to stirred cells along with 23 ml of a 1 x sucrose, 5 ml EDTA solution prepared by dissolving sucrose in 200 ml Tris and adding EDTA from a 100 mM stock, pH 7.5. After a 1:1 min-incubation, 45 ml of distilled water and 800 μl of phenylmethylsulfonyl fluoride (17.5 mM/ml) and N-tosyl-L-phenylalanine chloromethyl ketone (3.5 mg/ml) in ethanol were added. Cells were stirred for 30 min before the addition of 1.58 ml of 1 M MgSO₄ and deoxyribonuclease (10 mg dissolved in 200 ml Tris). After a 15 min incubation, 9.6 ml of 20% Triton X-100 were added, and the cells were incubated for an additional 15 min. The aforementioned steps were conducted at 4 °C. The resulting cell extract was centrifuged for 45 min at 144,000 × g in a Ti60 rotor at 4 °C. The resulting pellets were rehomogenized in 50 ml of ice-cold 50 mM Tris and 5 mM EDTA (Buffer A) to which 500 μl of the phenylmethylsulfonyl fluoride/N-tosyl-L-phenylalanine chloromethyl ketone solution had been added, and centrifugation was carried out as described above. All subsequent steps were conducted at room temperature. Centrifuge pellet fractions were further washed in 25 ml of Buffer A and 2% Triton X-100 and centrifuged at 20 °C under the same conditions described above. The resulting supernatant fractions were rehomogenized in 20 ml of Buffer A, brought to a final concentration of 1.5% Mega-10 by the addition of a 10% stock solution, and nutated for 20 min at 20 °C under the same conditions described above. The resulting supernatant fraction was saved, and BtuB was isolated as described below. The inclusion of 0.25% LIS during extraction effectively eliminated proteolytic degradation, if it was observed during the extraction of BtuB.

**BtuB Purification**—The supernatant fraction from the Mega-10 extract was diluted into 50 ml of Buffer A, 1% Mega-10, and 0.07% LIS and loaded onto a 10-ml DEAE-Sepharose CL-6B column. The column was washed with 20 ml of Buffer A, 0.35% Mega-10, and 0.05% LIS and eluted with 100 ml of 0−700 mM LiCl gradient in Buffer A, 0.33% Mega-10, and 0.05% LIS. Due to the intense UV absorption of LIS, the broad BtuB peak eluted throughout the latter half of the gradient was detected by Coomassie Blue staining (−15 min) of the fractions spotted onto Whatman #1 filter paper, followed by destaining with 20% methanol and 10% acetic acid. Pooled fractions were concentrated to approximately 2 ml with a Centricon-50, diluted into 40 ml of Buffer A and 1% Mega-10, and loaded onto a 2-ml DEAE-Sepharose CL-6B column. Detergent exchange was achieved by washing the column with 10 ml of Buffer A and 1% OG, followed by elution with 70 ml of 0−450 mM LiCl gradient in Buffer A and 1% OG. Pooled receptor fractions were concentrated to approximately 2 ml in a Centricon-50, and buffer exchange was carried out with a 10-ml Sephadex G-15 column equilibrated with 15 mM NaHPO₄ or 10 mM Tris and 1% OG, pH 7.5. Fractions were desalted and concentrated with a Centricron-100 and stored at −20 °C. BtuB purity was assessed by both SDS-PAGE and the presence of a unique ~74-kDa band visualized by silver staining (NH₄H₂O₂TSPD)-corresponding to that of the mature protein (19). Protein concentration was determined on a Varian Cary UV-visible spectrophotometer using an extinction coefficient at 280 nm of 140,000 M⁻¹ cm⁻¹ (25).

**SDS-PAGE and Gel Staining Methods—**BtuB fractions were routinely assayed for purity using a Tris-Tricine gel system (26) with stacking and resolving gels of 3 and 12.5%, respectively. Receptor conformation could be evaluated qualitatively by SDS-PAGE on a Phast Gel System (Pharmacia) using 12.5% acrylamide gels. Protein was detected by staining with either silver (27) or Coomassie Brilliant Blue (28). Bacterial lipopolysaccharide was resolved on the Tris-Tricine system described above and detected by carbohydrate-sensitive silver staining (29); development was terminated by a brief incubation in 20% Na₂S₂O₃. The LPS profile from the *E. coli* K12 derivative used for overexpression was evaluated by SDS-PAGE after microextraction and proteinase K digestion (30). The LPS content of BtuB preparations was estimated by densitometry of silver-stained gels with standard curves generated from *E. coli* EH100 LPS, which contains lip A and core sugars but lacks O-antigen, similar to the *E. coli* K12 derivative used for BtuB overexpression (Ref. 2; Fig. 2). Staining of LPS was conducted such that all species remained in the linear densitometric range.

**Circular Dichroism—**All measurements were acquired at room temperature using a JASCO-600 spectropolarimeter. Quartz cells with a path length of 0.2 mm were used for far UV measurements. Spectral parameters were as follows: (a) time constant, 2 s; (b) scan speed, 20 nm/min; (c) scanning increment, 0.1 nm; and (d) spectral bandwidth, 2 nm. Spectra were recorded in 15 mM NaHPO₄ and 1% OG titrated to pH 7.5 with NaOH at protein concentrations of 0.5, 0.55, 0.6, 0.75, and 1.5 mg/ml, and the buffer background was subtracted. For UV CD spectra of BtuB were analyzed for secondary structure information by the methods of Provencier and Glockner (31) and Chang et al. (32). The mean values of secondary structure content obtained from the two methods are reported.

**Analytical Ultracentrifugation—**Sedimentation equilibrium and sedimentation velocity measurements were performed in the Beckman OPTIMA XL-A analytical ultracentrifuge (AN60Ti rotor). BtuB dissolved in 50 mM Tris, 5 mM CaCl₂, and 1% OG, pH 7.5, was analyzed at concentrations of 0.5 mg/ml for velocity experiments and 0.35, 0.75, and 1.5 mg/ml for equilibrium experiments. Equilibrium and velocity runs were conducted in a 12-mm double sector cell at 20 °C, and absorbance was monitored at 275 nm. Velocity studies were performed at 50,000 rpm with sedimentation boundaries scanned at 10-min intervals. Sedimentation equilibrium studies were performed for 30 h at 10,000 rpm. The best fit to the sedimentation equilibrium data was analyzed with the program SEDEQ1B (A. P. Minton, minton@helix.nih.gov), and the velocity data were fit with the Svedberg (33) and the DCDT method (34). The partial specific volume of BtuB (0.726 cm³/g) was calculated from the amino acid composition. A partial specific volume of 0.859 cm³/g was used for octylglucoside (35). Molecular masses were generated for both the protein-detergent complex and the protein component alone, estimating the detergent bound to the protein at 0.5, 1.0, and 1.5 g/g (35). Sedimentation velocity experiments were conducted as described above after the preincubation of BtuB (0.5 mg/ml) with equimolar levels of colicin E₃ directly to demonstrate the formation of the BtuB-colicin E₃ complex. The binding of vitamin B₁₂ was measured in a similar fashion by simultaneously monitoring the sedimentation of protein (275 nm) and cyanocobalamin (358 nm) after the preincubation of the vitamin (15 or 30 μM) with BtuB (15 μM) solubilized in 10 mM Tris, 5 mM CaCl₂, and 1% OG, pH 7.5.

**Colicin Neutralization—**Receptor activity was also evaluated as the ability of purified BtuB to protect susceptible cells from the killing action of colicin E₁ or E₃. The sensitive *E. coli* strain K17D6 (pT77−) was grown to mid-log phase, and 150-μl aliquots were spread on 2YT Petri dish plates containing 100 μg/ml ampicillin. The plates were dried for 3 h at room temperature before the samples were washed with 15 mM NaHPO₄ or 10 mM Tris and 1% OG, pH 7.5. Colonies assayed for cytotoxicity were diluted to a final volume of 20 μl and preincubated for 20 min at room temperature, and 7.5 μl were subsequently applied to the plates described above. The plates were incubated overnight at 37 °C after the applied samples had dried, and colicin activity was evaluated as the ability to maintain a clear zone within a lawn of growing cells. All dilutions and reagents were prepared in 20 mM Tris and 1% OG, pH 7.5. Vitamin B₁₂ was added to BtuB 10 min before the addition of colicin in studies that assessed its effects on receptor function. Control experiments indicated that colicin was the agent responsible for cell killing under all conditions examined.

¹ The abbreviations used are: LPS, lipopolysaccharide; CD, circular dichroism; LIS, lithium 3,5-diodosalicylate; OG, β-octylglucopyranoside; PAGE, polyacrylamide gel electrophoresis; Tris, (tris [hydroxymethyl]aminomethane).
Purification and Characterization of BtuB

RESULTS

Overexpression and Purification—The use of E. coli TNE012 as an expression strain for BtuB purification has been described previously (17). However, an alternate procedure was necessary for the isolation of protein in quantities sufficient for characterization and crystallization trials. Overexpression from plasmid pJC3 coupled with the extraction of outer membranes with the nonionic detergent Mega-10 dramatically increased the amount of starting material for purification (25). Although prolonged cell growth may further enhance the expression levels, proteolytic degradation had been noted under such conditions, resulting in the detection of 20- and 40-kDa BtuB degradation products by SDS-PAGE. Co-purification of the degradation products likely resulted from a stable association between the fragments throughout the purification (data not shown).

The purity of BtuB after various stages of extraction and purification is shown in Fig. 1. Initial DEAE chromatography was facilitated by the presence of 0.05% LIS during column loading and elution, due to its ability to both inhibit observable proteolytic degradation and increase receptor purity by shifting the elution of BtuB to a later portion of the gradient. A second DEAE step was carried out for LIS removal, detergent exchange, and final purification. Pooled fractions from this second DEAE column contain a receptor species that is pure with respect to the protein (Fig. 1, lane 4) yet contains bound lipopolysaccharide (Fig. 2). Densitometry after SDS-PAGE and carbohydrate-sensitive silver staining using E. coli EH100 LPS for the generation of standard curves indicated that preparations contain approximately 2 ± 1 mol bound LPS/mol BtuB (data not shown).

Secondary Structure Analysis by Circular Dichroism and SDS-PAGE—Far UV CD spectra (190–250 nm) of BtuB solubilized in the nonionic detergent β-octylglucoside (Fig. 3A, ■) are characteristic of proteins integral to the bacterial outer membrane (5, 12), with a single minimum at 216 nm (arrow, a), a cross-over point at approximately 205 nm, and a relatively large peak of positive ellipticity centered around 195 nm. The averaging of the secondary structure values obtained from spectral analysis by the methods of Provencher and Glockner (31) and Chang et al. (32) indicates a predominantly β-sheet secondary structure (76 ± 4%; n = 3) and a small α-helical content (15 ± 3%; n = 3).

![Fig. 1. Extraction and purification of BtuB.](image)

![Fig. 2. Stoichiometric levels of lipopolysaccharide bound to BtuB after purification.](image)

![Fig. 3. Analysis of secondary structure by circular dichroism and SDS-PAGE.](image)
Purification and Characterization of BtuB

Fig. 4. Sedimentation equilibrium and sedimentation velocity analysis of octylglucoside-solubilized BtuB. BtuB solubilized in 15 mM NaH₂PO₄ and 1% OG, pH 7.5, was analyzed by sedimentation equilibrium (0.35 mg/ml) and sedimentation velocity (0.5 mg/ml). A, equilibrium analysis carried out for 30 h at 10,000 rpm at 20 °C with protein absorbance monitored at 275 nm. A, radial distribution of the protein at equilibrium. Inset, data in A plotted as ln A versus r², where r and A represent radial distance in centimeters and protein absorbance, respectively. B, distribution of S values from sedimentation velocity measured at 50,000 rpm at 20 °C with scans recorded at 10-min intervals.

Similar migration patterns are observed when BtuB is analyzed by SDS-PAGE, with the heat-treated receptor indicating a Mᵥ value close to the true molecular weight (Fig. 3B, lane 2); unheated samples underestimate the true Mᵥ value by approximately 20,000 (Fig. 3B, lane 3). The far UV CD spectrum of unheated receptor in the presence of OG/SDS mixed micelles (Fig. 3A, ---) demonstrates that secondary structure is retained in the presence of SDS, directly supporting the above interpretation of the migration patterns observed after SDS-PAGE. The incubation of BtuB at 90 °C in the presence of OG/SDS mixed micelles (Fig. 3A, ---) alters the secondary structure. The resulting spectrum has minima at approximately 208 and 222 nm (arrows, b and c), which is characteristic of some a-helical content, but lacks the positive peak of large amplitude centered at 190–195 nm (37).

Analytical Ultracentrifugation—Octylglucoside-solubilized BtuB was characterized by sedimentation equilibrium and sedimentation velocity to ascertain the oligomeric state and ensure the monodispersity of the protein-detergent complex. The analysis of equilibrium data yields plots of ln A versus r² that are linear from the meniscus to the cell bottom (Fig. 4A), indicating a homogenous preparation characterized by an aggregation state that is independent of sample concentration (0.35–1.5 mg/ml). Sample homogeneity is further demonstrated by the normal distribution of the sedimentation values obtained from the velocity studies (Ref. 34; Fig. 4B). The minor inhomogeneity observed at low S values is likely a result of light scattering from the slowly sedimenting octylglucoside micelles (Fig. 4B). From the slope of the ln A versus r² plot (Fig. 4A) and the sedimentation velocity data (Fig. 4B) analyzed according to Ref. 33, a sedimentation coefficient of 4.7 S and a molecular weight of 89,000 were calculated for the protein-detergent complex. The molecular weight of 89,000 includes an appreciable contribution from bound detergent. Calculations based on the assumption of a protein-detergent complex with detergent bound at levels of 1 g/g protein (13, 35) imply a contribution of approximately Mᵥ 60,000 for the protein component alone, indicating that BtuB was purified as a monomer. Similar calculations with estimates of detergent bound at a ratio of 0.5 g/g (approximately the molecular weight of a detergent micelle) or 1.5 g/g protein result in only a 20% difference in the calculated molecular weight, which is consistent with the conclusion that purified BtuB is monomeric.

Ligand Binding Characteristics of Purified BtuB—The initial binding to the receptor in the outer membrane is an obligatory step in colicin action. The formation of a high-affinity complex between colicin and purified receptor will decrease the concentration of free colicin, resulting in the protection of otherwise susceptible strains. The ligand binding characteristics of isolated BtuB were qualitatively evaluated by testing its ability to eliminate the cytotoxic effects of colicins E1 and E3. The preincubation of colicin E3 (16 nM) with increasing concentrations of BtuB eliminated observable cell killing once the receptor was provided in modest stoichiometric excess (22.5 nM; data not shown). In the absence of BtuB, cytotoxicity was apparent at a colicin E3 concentration of 0.16 nM, indicating that detergent-solubilized receptor retains high affinity for this particular ligand (Kᵥ 1 nM). In contrast, the preincubation of colicin E1 (17.5 nM) with a 15-fold molar excess of BtuB conferred no observable protection. Vitamin B₁₂ did not prevent the neutralization of colicin E3 by BtuB, even when it was present at approximately a 200-fold molar excess over the colicin.

The ability of detergent-solubilized BtuB to bind both vitamin B₁₂ and colicin E3 was directly demonstrated by sedimentation velocity analysis. Preincubation with stoichiometric levels of BtuB (15 µM) shifted the sedimentation profile of vitamin B₁₂ (Fig. 5B) to that observed for the isolated protein-detergent complex (Fig. 5A). Similar experiments carried out with a vitamin B₁₂: BtuB molar ratio of 2:1 revealed that approximately half of the vitamin does not sediment to any significant extent, as indicated by a nonsedimenting baseline equal to approximately half the total amplitudes (Fig. 5C). This implies a binding stoichiometry of approximately 1:1. In the case of colicin E3, the addition of equimolar concentrations of colicin (Fig. 6, traces e–h, measured at the same 10-min intervals as a–d) converts BtuB to a more rapidly migrating species with a sedimentation coefficient of 6.3 S. It is of interest to compare the sedimentation behavior of BtuB (4.7 S; Mᵥ 89,000) and the BtuB-colicin E3 complex (6.3 S; Mᵥ 150,000) upon the addition of colicin E3 (Mᵥ 61,000) to the Mᵥ 89,000 receptor-detergent complex with other characterized protein-detergent complexes of similar size: cytochrome c oxidase in C₈E₄₅ (4.1 S; Mᵥ ≈84,000; Ref. 38), (Na⁺ K⁺)-ATPase in C₁₂E₆ (6.5 S; Mᵥ 141,000; Ref. 39), and monomeric cytochrome b₅ complex in dodecylmaltoside (6.3 S; Mᵥ 149,000; Ref. 40).

DISCUSSION

Compared with previous studies (17, 18), the present study on the E. coli vitamin B₁₂ receptor has contributed the following: (a) the receptor has been purified to apparent homogeneity with greater than 50-fold higher yields; (b) the stoichiometry and homogeneity of LPS associated with BtuB have been determined after purification; (c) the secondary structure, which was previously based on prediction alone, has been determined by a comprehensive far UV circular dichroism analysis; and (d) the oligomeric state of isolated detergent-solubilized BtuB was rigorously determined.
chromatography and ultrafiltration (42). Repeated desalting with a Centricon-100 did not eliminate contaminating LPS from BtuB preparations. Similar experiments with *E. coli* EH100 LPS indicated that LPS should be removed under such conditions if it is not bound to the receptor (data not shown). The removal of associated LPS was previously shown to be necessary for the crystallization of porin (42). However, the low LPS:BtuB stoichiometry (2 ± 1:1), combined with an LPS species that appears homogenous in size (Fig. 2), raises the possibility that the bound LPS does not provide a major source of microheterogeneity.

Spectroscopic and SDS-PAGE Analysis of the Secondary Structure and Conformational Changes—Sequence analysis of both porins and TonB-dependent receptors reveals an absence of hydrophobic stretches of sufficient length to span the bilayer as independent α-helices. Circular dichroism analysis of BtuB in the presence of the nonionic detergent octylglucoside and octylglucoside/SDS mixed micelles indicated a predominantly β-sheet secondary structure with low α-helical content, providing the first spectroscopic characterization of the BtuB secondary structure.

Resistance to denaturation by SDS results in a compact, faster-migrating species during SDS-PAGE if the samples are not boiled before electrophoresis, providing a simple, rapid means of evaluating receptor conformation. This property may prove of particular value for crystallization trials, allowing insights into the effects of prolonged storage and environmental conditions on protein conformation. The CD spectrum of BtuB obtained after heat denaturation in the presence of OG/SDS mixed micelles is peculiar. The expectation of a single peak of negative ellipticity centered between 195 and 200 nm for a fully denatured protein (37), coupled with the relatively weak ellipticity of the two negative transitions and the well-characterized ability of SDS to induce α-helix formation (43), suggests that this spectrum may arise from a largely unordered species that adopts a certain degree of α-helical secondary structure upon heat denaturation in the presence of SDS. Because unordered proteins are not represented in the algorithms used to evaluate BtuB secondary structure content, it would be of interest to analyze such spectra with algorithms that include unordered proteins in the reference set (44).

**Oligomeric State of Purified BtuB**—In contrast to the situation with a variety of bacterial porins that associate to form highly stable trimers, the literature concerning the oligomeric state of the TonB-dependent receptors FhuA, FepA, and BtuB is less clear. The characterization of detergent-solubilized FhuA by sedimentation equilibrium and sedimentation velocity indicated that it was purified as a monomer (13, 36), although further analysis of the ligand binding properties and the ability to cross-link a certain fraction protein into dimers and trimers led to the proposal of transient oligomerization in solution (36). For the TonB-dependent receptor FepA, Western blot analysis of outer membrane fractions after solubilization with lithium dodecyl sulfate and LDS-PAGE raised the possibility that it was extracted as a mixed population of monomers and trimers (15), although such experiments cannot rule out the possibility of stable hetero-oligomer formation. For BtuB, the possibility of quaternary structure or hetero-oligomer formation within the context of native outer membranes remains to be explored. However, the centrifugation analysis carried out in the present study clearly indicate that the receptor was purified as a monomer. A combination of BtuB and OmpF serving as the optimal colicin A receptor (45) suggests at least a transient association between these two species during the colicin translocation process.

**Ligand Binding Characteristics of Purified BtuB**—The iso-

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**Purified BtuB: A Potential Model System for Membrane Protein Structure Determination and Receptor-Ligand Interactions**—The availability of a rapid and convenient procedure for the purification of BtuB makes it an attractive system to study the characteristics of the TonB-dependent receptor family as well as the general principles of membrane protein structure, receptor-ligand interactions, and energy-dependent transport phenomena. The isolation scheme presented in this work not only results in higher yields but also avoids the laborious and unreliable process of affinity chromatography on colicin E3-Sepharose (17). The purified receptor contains LPS, as previously observed during the purification of other outer membrane proteins (36, 41). It will be of interest to evaluate the effects of delipidation on receptor properties because this species appears tightly bound relative to the situation with bacterial porin in which LPS could be removed through ion-exchange.
Purification and Characterization of BtuB

The inability of purified receptor to neutralize colicin E1 is consistent with prior observations suggesting that the affinity of BtuB for vitamin B12 is somewhat decreased after purification. Preliminary sedimentation velocity studies indicate that colicin E1 and vitamin B12 bind to BtuB in a competitive fashion (data not shown), further suggesting that the aforementioned ligands either share a common binding site or that the binding of one ligand results in a loss of determinants required for the recognition of a second ligand at a discrete site.

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