**Purification and Characterization of Monomeric *Escherichia coli* Vitamin B12Receptor with High Affinity for Colicin E**

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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.

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