

Native clathrin self-associates to form a bimodal and physiological collection of closed lattices. *H. T. Pretorius, P. K. Nandi, R. E. Lippoldt, J. H. Keen, I. Pastan, H. Edelhoch, and M. L. Johnson. National Institutes of Health, Bethesda, MD.*

Receptor-mediated endocytosis plays an important role in the internalization into cells of cholesterol-rich, low density lipoprotein of human serum, alpha-2 macroglobulin, insulin, epidermal growth factor, and probably many other substances. In some cases these involve the particular type of pinocytosis mediated by coated pits or coated vesicles. The protein coat on the coated vesicle is predominantly formed by the protein clathrin. Clathrin will self-associate in vitro into empty vesicular appearing structures without any lipid present. The isolation procedure used for clathrin in our studies did not use sodium dodecyl sulfate (SDS), urea, or other denaturants but instead used an extraction with 0.01 M Tris-HCl buffer followed by gel permeation chromatography on Sepharose CL4B columns. Clathrin is eluted in the second protein peak in the effluent. The clathrin moves on SDS gels at approximately 175,000 molecular weight. Overloading the gels shows that there are some other proteins present as well in the second peak. Homogeneous clathrin was obtained from reduced and alkylated material using additional gel permeation chromatography in the presence of 6 M guanidine hydrochloride. Monitoring of tryptophyl fluorescence in the eluate from such columns shows a major clathrin peak. After dialysis to remove the guanidine hydrochloride, SDS gel electrophoresis gives a single band for clathrin. Equilibrium sedi-

mentation experiments and gel electrophoresis studies both gave a molecular weight for the clathrin monomer of  $170,000 \pm 26,000$ . Undenatured clathrin from the peak II of the first Sepharose column is stable over a large range of pH, temperature, and ionic strength. Equilibrium sedimentation studies of this material were consistent with a single species of molecular weight  $610,000 \pm 30,000$ . Sedimentation velocity gave a sedimentation constant of 8.2S. The frictional ratio is 3.1, indicating a hydrodynamically asymmetrical structure. The proportion of alpha helix in this clathrin preparation was estimated to be approximately 50% with 15% beta-pleated sheet conformation using computer analysis of circular dichroism (CD) spectra. These data suggest that 8.2S clathrin has a semirigid structure, probably with some domains that are rigid and interdomain regions that are flexible. The less rigid areas may be necessary for the formation of the various angles in the polygonal structures seen in the coated vesicles at high magnification.

The 8.2S clathrin polymerizes when the ionic strength is reduced to form two high molecular weight species in sedimentation velocity experiments, one near 150S and the other near 300S. Electron microscopic studies of the rat vas deferens by Friend and Farguhar (*J. Cell Biol.* 35: 357-376; 1967) and also in coated vesicle preparation from adrenal medulla (*Proc. Nat. Acad. Sci. USA* 73: 1255-1259; 1976) demonstrate two types of coated vesicles, when lipid is present, one large and one small type.

#### References

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2. Pearse, B. M. F. On the structural and functional components of coated vesicles. *J. Mol. Biol.* 126: 803-812; 1978.