

Molecular Characterization of Human Clathrin[†]

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ABSTRACT: Clathrin extracted from coated vesicles at pH 8.0 sediments as a single boundary with 8.1S sedimentation constant ($s_{20,w}^0$) of 8.1 ± 0.1 S. Sedimentation equilibrium gave a molecular weight (M_r) of $610\,000 \pm 30\,000$. The clathrin frictional ratio (pH 7.5) computed from $s_{20,w}^0$ and M_r is very large, i.e., 3.06 ± 0.18 . Analysis of the circular dichroic spectrum in the far-ultraviolet showed that about half of the peptide residues are in a α -helical conformation. The molecular weight of a preparation of clathrin purified to homogeneity on a Sepharose CL-4B column in 6 M guanidine hydrochloride was $170\,000 \pm 26\,000$ by sedimentation equilibrium, which is in agreement with the values we and others obtained by sodium dodecyl sulfate gel electrophoresis. The 8.1S clathrin species may be regarded as the "native" promoter since (1) it is extracted from coated vesicles by an extremely mild procedure, (2) it is stable over considerable ranges of pH,

temperature, and ionic strength, and (3) it readily polymerizes into characteristic closed lattice structures resembling those observed in coated vesicles in the electron microscope. The 8.1S clathrin molecule self-associates at pH 6.3 to form two very high molecular weight species with average sedimentation coefficients of 150 and 300 S. The sedimenting boundaries of both of these species have been analyzed to reveal their molecular heterogeneity. The two species observed by sedimentation velocity may correspond to the two sizes of coated vesicles previously reported to be present in some cells when observed by electron microscopy. Analysis of the sedimentation pattern in the ultracentrifuge also gives the amount of unreacted 8.1S clathrin from which the yield of polymerizable clathrin is obtainable. This methodology can therefore be employed to estimate the quality of the 8.1S preparation of clathrin and thereby affords an assay of its activity.