Oligomeric Structure of Mammalian Purine Nucleoside Phosphorylase in Solution Determined by Analytical Ultracentrifugation

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The influence of phosphate, ionic strength, temperature and enzyme concentration on the oligomeric structure of calf spleen purine nucleoside phosphorylase (PNP) in solution was studied by analytical ultracentrifugation methods. Sedimentation equilibrium analysis used to directly determine the enzyme molecular mass revealed a trimeric molecule with M_r = (90.6 ± 2.1) kDa, regardless the conditions investigated: protein concentration in the range 0.02–1.0 mg/ml, presence of up to 100 mM phosphate and up to 200 mM NaCl, temperature in the range 4–25 °C. The sedimentation coefficient (6.04 ± 0.02) S, together with the diffusion coefficient (6.15 ± 0.11) 10^{-7} cm^2/s, both values obtained from the classic sedimentation velocity method at 1.0 mg/ml PNP concentration in 20 mM Hepes, pH 7.0, yielded a molecular mass of (90.2 ± 1.6) kDa as expected for the trimeric enzyme molecule. Moreover, as shown by active enzyme sedimentation, calf spleen PNP remained trimeric even at low protein concentrations (1 μg/ml). Hence in solution, similar like in the crystalline state, calf spleen PNP is a homotrimer and previous suggestions for dissociation of this enzyme into more active monomers, upon dilution of the enzyme or addition of phosphate, are incorrect.

Key words: Purine Nucleoside Phosphorylase (PNP), Oligomeric State, Analytical Ultracentrifugation