Interactions of Calf Spleen Purine Nucleoside Phosphorylase with 8-Azaguanine, and a Bisubstrate Analogue Inhibitor: Implications for the Reaction Mechanism

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Interactions of calf spleen purine nucleoside phosphorylase (PNP) with a non-typical substrate, 8-azaguanine (8-azaG), and a bisubstrate analogue inhibitor, 9-(2-phosphonylmethoxyethyl)-8-azaguanine (PME-azaG), were investigated by means of steady-state fluorescence spectroscopy. Both 8-azaG and PME-azaG form fluorescent complexes with the enzyme, and dissociation constants are comparable to the appropriate parameters (K_m or K_i) obtained from kinetic measurements. PME-azaG inhibits both the phosphorolytic and synthetic pathway of the reaction in a competitive mode. The complex of 8-azaG with PNP is much weaker than the previously reported Gua-PNP complex, and its dissociation constant increases at pH > 7, where 8-azaG exists predominantly as the monoanion ($pK_a \approx 6.5$). The fluorescence difference spectrum of the PNP/8-azaG complex points to participation of the N(7)H or/and N(8)H tautomers of the neutral substrate, and the 9-(2-phosphonylmethoxyethyl) derivative also exists as a neutral species in the complex with PNP. The latter conclusion is based on spectral characteristics of the PNP/PME-azaG complex, confirmed by fluorimetric determination of dissociation constants, which are virtually pH-independent in the range 6–7. These findings testify to involvement of the neutral purine molecule, and not its monoanion, as the substrate in the reverse, synthetic reaction. It is proposed that, in the reverse reaction pathway, the natural purine substrate is bound to the enzyme as the neutral N(7)H tautomer, which is responsible for the reported strong fluorescence of the guanine-PNP complex.

Key words: Purine Nucleoside Phosphorylase, 8-Azaguanine, Fluorescence Titration