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Partial purification and properties of thiamine pyrophosphokinase from pig brain

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Abstract

Pig brain thiamine pyrophosphokinase (ATP: thiamine pyrophosphotransferase, EC 2.7.6.2) was purified 260-fold over extracts of brain acetone powder. A direct, radiometric assay was used to follow the purification. By isoelectric focusing, the purified enzyme appeared to have an isoionic point of approx. pH 4.2, but these preparations were still not homogeneous by disc-gel electrophoresis nor by analytical ultracentrifugation.

The purified enzyme has a broad pH optimum extending from pH 8.3 to 9.3 in 0.028 M phosphate/glycylglycine buffers. For optimal enzymatic activity, the ratio of magnesium to ATP must be fixed at 0.6, which suggests that for this ATP-pyrophosphoryl transfer reaction, the enzymatically preferred reactant may be $Mg(ATP)_2^{6â^,}$. A preliminary study

â€œping-pongâ€ mechanism, on this basis, dissociation constants for ATP_t and for thiamine were evaluated. Pyrithiamine, butylthiamine, ethylthiamine, and oxythiamine appeared to be competitive inhibitors withr respect to thiamaine as the variable substrate, and their inhibitor dissociation constants were calculated. The relatively poor affinity of oxythiamine to the enzyme emphasizes the 4-amino group in the pyrimidine ring as one of the specificity requirements for thiamine pyrophosphokinase. Preliminary values for the apparent equilibrium coefficient of the thiamine pyrophosphokinase-catalyzed reaction, in terms of total species, has been approximated at several initial concentrations of reactants: e.g. $K'_{eq,app}$ =

[AMPt]eq[TDPt]eq[ATPt]eq[TH]eq=0.9Â·10â^'5 â^' 1.6Â·10â^'5,at initial[ATPt]initial=[Mgt]initial0.6= 9.66 Â· 10â^'3M

and $[Th]_{initial}$ = 1 Â· $10^{â^'6}$ and 2 Â· $10^{â^'6}$ M, respectively, where TDP, Th, t and eq represent thiamine diphosphate, thiamine, total concentration and â€œeqâ€, equilibrium concentration, respectively.

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Abbreviations

a subscript â€œtâ€, is used to represent total concentrations; a subscript â€œeqâ€, equilibrium concentrations; a subscript â€œfâ€, for forward reaction; a subscript â€œappâ€, an apparent value

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