THE STRUCTURE OF THE METAL-NUCLEOTIDE COMPLEX SUBSTRATE FOR YEAST HEXOKINASE

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SUMMARY

A comparison has been made of the kinetic parameters obtained with yeast hexokinase using Mg^{2+} or Ni^{2+} as metal ion activator and ATP or tubercidin-5'-triphosphate as nucleotide substrate. It is concluded that the relative specificity of the enzyme for $MgATP^{2-}$ does not involve a contribution arising from an interaction of the metal ion with the purine ring of the nucleotide.

It has been proposed, on the basis of kinetic information, that the nucleotide substrate for yeast hexokinase is $MgATP^{2-}$ (1-4). This enzyme will also catalyze the formation of glucose-6-phosphate when other divalent metal ions and other nucleoside triphosphates are used. It has been shown that, in addition to Mg^{2+}, the reaction is stimulated by Mn^{2+} (5,6) and Ca^{2+} (5,7). Both naturally occurring and synthetic analogs of ATP can serve as the phosphate donor (8-10).

There are at least two obvious explanations for the requirement of a metal-nucleotide complex as substrate. The structure of the complex in which the phosphate bound metal interacts with the purine ring may provide recognition features for binding and/or reactive areas for catalysis. A second possibility is that only the binding of the metal to the phosphates is essential. The purpose of the experiments described below is to choose between these two possibilities by taking advantage of what is known about the structure of metal-nucleotide complexes in aqueous solution. There is a good deal of evidence, primarily from nmr studies, that divalent cations react with both the adenine ring and the phosphate groups of ATP. A range of possible interactions exist depending on the specific metal ion used. Divalent metal ions have been reported to complex with all three phosphates of ATP (Mn^{2+}, Co^{2+}, Ni^{2+}) or with only the β and γ phosphates (Mg^{2+}, Ca^{2+}, Zn^{2+}) (11-13). More recently, phosphorus

Fourier transform analysis has revealed that Mg^{2+} and Ca^{2+} also react with all three phosphates of ATP (14). While Ni^{2+} and Co^{2+} and possibly Mn^{2+} interact via a bridging water molecule with the N-7 of the adenine ring of ATP (15,16), Mg^{2+}, but not Ca^{2+}, reportedly interacts in an as yet unspecified manner with the N-1 or N-3 positions (14).

The rationale of the experiments below is to compare the kinetic parameters obtained with two different nucleotides and two different divalent metal ions. If the only important feature of the complex from the enzymatic point of view is the metal-phosphate interaction, then the fact that different metal ions interact differently with the ring should have no bearing on the results. If, on the other hand, the interaction of the metal ion with the purine ring is important, then comparing an N-7 outer sphere complex with an N-1/N-3 complex might show large differences. In order to make the comparison we selected ATP and tubercidin -5'-triphosphate (TuTP) as nucleotides and Mg^{2+} and Ni^{2+} as divalent metal ions. TuTP has a carbon atom in position 7 of the ring in place of the nitrogen in adenine. From nmr studies we have found that in the Ni-TuTP complex the ring interaction is at N-1 or N-3 (15). If the metal-ring interaction is important then Ni-ATP and Ni-TuTP should be quite different. Since Mg^{2+} appears to interact with ATP at N-1 and N-3 (14) and probably forms similar complexes in the case of MgATP and Mg-TuTP (17), these two complexes should not be too different. We have previously reported that TuTP is almost equivalent to ATP as a substrate for yeast hexokinase when Mg^{2+} is the activating metal ion (10).

MATERIALS AND METHODS

Yeast hexokinase (Type C-300) and glucose-6-phosphate dehydrogenase (Type XV) were purchased from Sigma.

The preparation of the enzymes and reagents, and the performance of the assay was a previously described (10). The stability constant for $MgATP^{2-}$ used for calculations was 48,000 (18). This is in excellent agreement with the value of 50,000 obtained by correcting the data of Frey and Stuehr (19)

TABLE 1

Nucleotide	Metal Ion	K_m (mM)	V_{max} (μmoles$\cdot$min$^{-1}\cdot$mg^{-1})
ATP	Mg^{2+}	0.28 $\pm$ .03	356 $\pm$ 13
ATP	Ni^{2+}	0.16 $\pm$ .01	81 $\pm$ 2
TuTP	Mg^{2+}	0.23 $\pm$ .03	306 $\pm$ 18
TuTP	Ni^{2+}	0.11 $\pm$ .01	80 $\pm$ 3

The assay medium contained 50 mM HEPES (N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid) neutralized with tetramethylammonium hydroxide, 50 mM tetramethylammonium nitrate, 9 mM glucose, 0.4 mM NADP, 3.2 units glucose-6-phosphate dehydrogenase and triple distilled water to a final volume of 320 μl and a final pH of 8.0. After a 5 min preincubation at 25° the reaction was started by the addition of a 5 μl aliquot containing 0.05 unit yeast hexokinase. For each analysis 9 concentrations of metal nucleotide ranging from 0.08-0.9 mM were used. The values given are the mean $\pm$ S.D.

for temperature and potassium binding. The temperature correction was made by using the van't Hoff equation and a value of 2600 for ΔH (20) while the value for the binding constant of KATP at an ionic strength of 0.1 M was taken as 30 (19). A value of 225,000 was used for the stability constant of $NiATP^{2-}$. This was obtained by correcting the value of Frey and Stuehr (19) for temperature and potassium binding. Experiments with a copper specific electrode showed there was no interaction betwen Cu^{2+} and the HEPES buffer. Since Cu^{2+} interactions are stronger with nitrogen containing ligands than Ni^{2+} it was assumed there was also no interaction between Ni^{2+} and HEPES.

RESULTS AND DISCUSSION

The results are shown in Table 1. The main point is that alterations in kinetic parameters result from changes in metal ion and not from the metal ion-nucleotide combination. The results were identical when the concentration of free metal ion was varied from 5-150 μmolar. The conclusion drawn from these experiments is that the important feature of the metal-nucleotide complex as far as the enzymatic reaction is concerned is the interaction of the metal ion with the phosphate groups and not with the ring. It is possible that the inter-

action of the phosphate bound metal ion with the nucleotide ring is restricted on the enzyme-substrate complex. Such a situation could arise if an interaction of the nucleotide ring with the enzyme alters the hardness-softness properties of the potential sites on the ring and therefore their ability to coordinate with the metal-phosphate complex (17). One potentially important parameter involved in the hardness-softness property is the microscopic dielectric constant which is known to undergo variation during the course of many enzymatic reactions.

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