Biochimica et Biophysica Acta, 615 (1980) 103—112

BBA 69091

FURTHER KINETIC CHARACTERIZATION OF THE NON-ALLOSTERIC PHOSPHOFRUCTOKINASE FROM *ESCHERICHIA COLI* K-12

KEN N. EWINGS and HORST W. DOELLE

Department of Microbiology, University of Queensland, St. Lucia, Queensland 4067, (Australia)

(Received January 11th, 1980)

Key words: Phosphofructokinase; Allostery; (E. coli K-12)

Summary

The labile non-allosteric form of phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) was purified to a specific activity of 107 U/mg (2078-fold) from aerobic cultures of *Escherichia coli* K-12. The enzyme has an isoelectric point (p*I*) of 5.1, a native molecular weight of 67 000 ± 3000 and a subunit weight of 34 000 ± 400.

A number of divalent metal ions can substitute for Mg^{2+} in the enzyme reaction in decreasing order $Mn^{2+} > Mg^{2+} > Co^{2+} > Ca^{2+}$. In the presence of excess Mg^{2+}, nucleotides do not affect the K_m for fructose 6-phosphate with a value of 0.042 mM. The order of efficiency for nucleotides to act as phosphoryl donors is ATP > ITP > GTP > UTP > CTP. This remains unchanged in the presence of excess Mn^{2+}, but V is increased 2.4-fold with ATP. A 2 : 1 ratio of Mn^{2+}/nucleotide 5′-triphosphate produced an equivalent dissociation constant of 1.1 mM for all nucleotides, which was markedly decreased at a high Mn^{2+} level. The rate of enzyme catalysis was found to be dependent on the concentration of $MnATP^{2-}$. Mn^{2+} at non-limiting values does affect the binding of fructose 6-phosphate to the enzyme.

Introduction

Bacteria are known to produce a non-allosteric form of phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11), a situation which is rare in eucaryotes. Organisms reported to synthesize enzymes exhibiting non-sigmoidal kinetics in response to fructose 6-phosphate are *Lactobacillus casei* [1], *Lactobacillus plantarum* [1,2] and *Arthrobacter crystallopoietes* [3]. The existence of both an allosteric and a non-allosteric form appears to be unique to *Escherichia coli* [4,5].

Since the major allosteric enzyme has recently come into question with regard to its role as an important control point in glycolysis in *E. coli* [6], it was of interest to examine the role of the second form in relation to possible as yet unknown metabolite control [7—9]. This enzyme constitutes less than 10% of the total phosphofructokinase production in *E. coli* and is not subject to synthetic repression, which is in contrast to that of the major allosteric protein [7,10]. The non-allosteric enzyme has also been noted for its instability [8]. Although an in vitro interconvertible restoration of activity can be achieved by a combination of substrate-metabolite effectors [11], this property alone cannot be a true reflection on the in vivo situation.

This paper presents further kinetic characterization of the second phosphofructokinase isozyme isolated from a wild-type strain of *E. coli* K-12. Evidence suggests that there is a tendency for non selection between nucleotide triphosphates in the presence of Mn^{2+} and in particular a preference for $MnATP^{2-}$ as a substrate.

Materials and Methods

Growth of organism. E. coli K-12 (UQM 332) was grown under aerobic continuous cultivation as described earlier [12,13]. Cells were harvested by trapping the overflow in chloramphenicol (50 μg/ml) at 2°C and subjecting the culture to centrifugation at 10 000 × *g*. The pellet was washed twice with 0.9% NaCl and finally with buffer 1 containing 10 mM Tris-HCl (pH 8.2)/1 mM $MgCl_2$/0.2 mM EDTA/10 mM 2-mercaptoethanol/5% (v/v) glycerol. Pellets were stored at −20°C until required.

The protein content of the enzyme preparations was estimated according to Lowry et al. [14].

Enzyme assay. For the purification of the enzyme, an assay system was used, which utilizes the insensitivity of this protein towards phospho*enol*pyruvate as initially described by Kotlarz et al. [7]: 0.1 M Tris-HCl (pH 8.4), 0.2 M $MgCl_2$, 2 mM ATP, 0.3 mM fructose 6-phosphate, 3 mM phospho*enol*pyruvate, 0.2 mM $NADH_2$, 30 μg/ml aldolase, 5 μg/ml triosephosphate isomerase, and 30 μg/ml glycerol phosphate dehydrogenase in a total of 2.0 ml. Assay blanks were performed throughout the isolation procedure to detect levels of NADH oxidase, phosphatase, fructose bisphosphatase and adenylate kinase. A unit enzyme was defined as the amount of enzyme that catalyzes the formation of 1 μmol of fructose 1,6-bisphosphate per min at 25°C.

For the kinetic investigations the phosphofructokinase activity was determined according to Vinuela et al. [15]. The standard reaction mixture (2 ml) contained 0.01 M Tris-HCl (pH 8.4), 0.2 mM $NADH_2$ and 0.1 ml auxiliary enzymes. Fructose 6-phosphate, $MgATP^{2-}$ and $MnATP^{2-}$ were added. All enzymes were dialyzed for 4 h against two 2-volume changes of 0.01 M Tris-HCl (pH 8.4) and 0.2 mM EDTA at 4°C. The addition of Mn^{2+} to the assays was made from freshly-prepared stock-solutions of acidified $MnCl_2$ containing 1 mM 2-mercaptoethanol as a precaution against the formation of higher manganese oxidation states. $MgATP^{2-}$ and $MnATP^{2-}$ concentrations were made by mixing the metal chloride salts and Na_2ATP. The concentrations of the metal-nucleotide complexes and free divalent metal cations were calculated

from the reported stability constants ($2 \cdot 10^4\ M^{-1}$) for the $MgATP^{2-}$ complex [16,17] and for the $MnATP^{2-}$ complex ($1 \cdot 10^5\ M^{-1}$) [18] using the method of Ahlers [19]. A minimal enzyme concentration of 2 μg was used to ensure accurate rate constants [20]. The pure enzyme preparation was supplemented with bovine serum albumin (20 μg/ml) prior to kinetic assays to maintain stability when not stored at −20°C. The reaction was started by the addition of the enzyme. Enzyme activity was measured by monitoring the rate of $NADH_2$ oxidation at 340 nm with a Unicam SP-8000 ultraviolet recording spectrophotometer. The reaction velocity was expressed in enzyme units (μmol fructose bisphosphate/min) at 25°C.

Evaluation and treatment of kinetic data. The double-reciprocal plots of the concentration-velocity curves were calculated with the least-squares-fit method on an Olivetti Programma desk-top computer. The calculation of the dissociation constants (K_m) and V were obtained from the regression line which gave the ordinate axis intercept as $1/V$. Reversal of the reciprocal values on the ordinate and abscissa resulted in a value of $1/K_m$. These two separate computations of the regression line provided a check on the accuracy of the data input from equivalent correlation coefficients. Equal weight was placed on all data. Enzyme velocities used were mean values of triplicate experiments performed at each calculated species concentration.

Enzyme purification. A scheme of similar protocol to that of Kotlarz et al. [8] with minor modifications was used to purify the enzyme. The addition of a heat precipitation step at pH 5.0 and the use of preparative isoelectric focusing resulted in a 14-fold increase in the specific activity of the enzyme. Hydroxyapatite chromatography and gel filtration on Sephacryl S-200 made up the final stages of the isolation which produced a homogeneous protein of specific activity 107 U/mg. A summary of the enzyme purification is presented in Table I.

Native molecular weights were obtained by gel filtration on Sephacryl S-200

TABLE I

SUMMARY OF THE PURIFICATION OF THE NON-ALLOSTERIC FORM OF PHOSPHOFRUCTOKINASE FROM THE WILD-TYPE *E. coli* K12

Purification steps	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification	Yield (%)
Extract	828.0	16 100	0.051	—	100
Streptomycin sulphate precipitate	806.0	10 000	0.081	1.6	97
$(NH_4)_2SO_4$ fractionation (40—70%)	718.0	4 595	0.16	3.1	87
Acid/heat precipitate pH 5.0/50°C	710.0	3 353	0.21	4.1	86
DEAE-cellulose chromatography	497.0	1 718	0.29	5.7	60
Isoelectric focusing	348.0	86	4.0	79.8	42
Hydroxyapatite chromatography	86.8	7	12.4	243.0	10
Sephacryl S-200 chromatography	60.0	0.6	101.0	1 980.0	7
Heat treatment (65°C)	59.0	0.55	107.0	2 098.0	7

(1.6 × 95 cm) according to Andrews [21] and the void volume was defined with Blue Dextran. Protein standards used were: cytochrome *c*, 12 400; chymotrypsinogen, 25 000; ovalbumin, 45 000; bovine serum albumin, 67 000; alkaline phosphatase, (*E. coli*), 80 000; lactate dehydrogenase, 142 000; aldolase, 160 000 and catalase, 240 000. Sucrose density-gradient centrifugation was used to determine the molecular weight according to Martin and Ames [22]. Standard proteins used were malate dehydrogenase, 70 000; lactate dehydrogenase, 142 000 and pyruvate kinase, 237 000.

Protomer molecular weights were determined using the technique of Weber and Osborne [23]. Standard proteins used were lysozyme, 14 000; β-lactoglobulin, 18 400; trypsinogen, 24 000; pepsin, 34 700; ovalbumin, 45 000 and serum bovine albumin, 67 000.

Analytical isoelectric focusing was performed using plate gels of 5% acrylamide with a 3% cross-linkage on an LKB multiphor. The pH range of 4—6.5 was achieved with 0.2% (w/v) solutions of Pharmalyte ampholines employing histidine (0.1 M) and glutamic acid (0.03 M) as the cathode and anode electrolytes, respectively. Focusing was carried out for 5 h to a maximum of 1450 V. The gel was fixed and stained directly for proteins using procedure B of Vesterberg [24].

Results

Isoelectric point

Isoelectric focusing in the pH range between 4.0 and 6.5 produced two closely spaced bands at pH 5.1 ± 0.03 and 4°C.

pH Optimum

The enzyme assay was carried out over the pH range of 4.0 to 10.0. The non-allosteric form of phosphofructokinase exhibited two pH optima at 6.8 and 8.4, whereby the acidic peak contained 25% of the total enzyme activity. All further kinetic studies were carried out at pH 8.4.

Kinetic properties

Divalent metal ion requirement. The ability of various divalent metal ions to substitute for Mg^{2+} is illustrated in Table II. To detect either inhibition or activation from the uncomplexed metal ions, two concentrations of the metal salts were used at 1.0 and 10.0 mM in the presence of 1.0 mM ATP. Metal-nucleotide complexes were not formed in the presence of Cd^{2+}, Ba^{2+} or Fe^{2+}, and Ca^{2+} proved to be a poor substitute for Mg^{2+}. Enzyme velocity is increased at 10.0 mM for Mn^{2+}, Mg^{2+} and Co^{2+}, but is markedly enhanced at both 1.0 and 10.0 mM in the presence of Mn^{2+}. In Fig. 1, enzyme velocity was measured against increasing amounts of Mn^{2+}, Mg^{2+}, Co^{2+} and Ca^{2+} at 1.0 mM ATP. Reciprocal plots of this data yielded apparent dissociation constants of 0.29 and 0.70 mM for $MgATP^{2-}$ and $MnATP^{2-}$, respectively. Optimal activity occurred at metal/ATP ratios of 4 : 1 and 9 : 1 for Mn^{2+} and Mg^{2+}, respectively. There is no further inhibition or activation from either of the divalent metal ions beyond the region of these concentration ratios.

Substrate specificity. An examination of the effect on the dissociation

TABLE II

ABILITY OF DIVALENT METAL IONS TO REPLACE Mg^{2+} FOR THE NON-ALLOSTERIC FORM OF PHOSPHOFRUCTOKINASE

Assays were performed in the presence of 1.0 mM fructose 6-phosphate and 1.0 mM ATP

Metal salt	% Relative activity *	
	1.0 mM	10.0 mM
$MgCl_2$	34	100
$MnCl_2$	102	305
$CoCl_2$	24	61
$CaCl_2$	5	14
$CdCl_2$	0	0
$BaCl_2$	0	0
$FeCl_2$	0	0

* Values are expressed as a percentage of the activity measured with 10 mM $MgCl_2$.

constant for fructose 6-phosphate from the nucleoside trisphosphates ATP, CTP, GTP, ITP and UTP is given in Table III. At a fixed ratio of Mg^{2+}/nucleotide 5′-triphosphate of 10 : 1, there are only minor changes in the K_m for fructose 6-phosphate at both high (2.0 mM) and low (0.2 mM) concentrations of the nucleosides, indicating that the nature of the phosphoryl donor does not affect the binding of the hexose phosphate. However, as presented in Table IV, there are differences in the ability of the nucleoside triphosphates to act as substrates. If the relative velocities are taken as a percentage of the activity at 0.2 mM ATP, the latter is the most efficient substrate in the presence of either Mg^{2+} or Mn^{2+}. At a 2.0 mM nucleoside concentration, the same trend is apparent although significant velocity increases are evident with the fixed Mn^{2+}/nucleotide 5′-triphosphate ratio of 4 : 1.

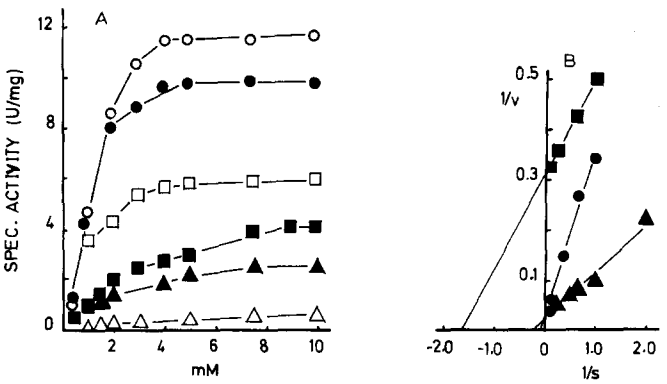


Fig. 1. Effect of divalent metal ion concentrations on the non-allosteric forms of phosphofructokinase from *E. coli* K-12. Assays were performed as described in the text. A: Saturation curves with 1 mM fructose 6-phosphate and the concentrations of metal ions as indicated: ○———○, 1 mM Mn/ATP; ●———●, 0.5 mM Mn/ATP; □———□, 0.1 mM Mn/ATP; ■———■, 1 mM Mg/ATP; ▲———▲, 1 mM Co/ATP and △———△, 1 mM Ca/ATP. B: reciprocal plots of divalent metal ion concentration on enzyme activity. Results were calculated from a series of experiments similar to those shown in A to ascertain $K_{diss.}$ for each divalent metal ion complex: ▲———▲, Co/ATP; ●———●, Mn/ATP and ■———■, Mg/ATP.

TABLE III

APPARENT MICHAELIS-MENTEN CONSTANTS FOR FRUCTOSE 6-PHOSPHATE OF THE NON-ALLOSTERIC FORM OF PHOSPHOFRUCTOKINASE IN THE PRESENCE OF VARIOUS NUCLEOSIDES

Assays were carried out in presence of Mg^{2+} at a fixed ratio of 10 : 1 (Mg^{2+}/nucleotide 5'-triphosphate). Values were expressed as mM

Nucleotide	2 mM	0.2 mM
ATP	0.042	0.043
CTP	0.039	0.041
GTP	0.042	0.056
UTP	0.037	0.042
ITP	0.041	0.050

Dissociation constants for the nucleosides ATP, GTP, and ITP show an interesting trend in specificity. In Table V, the K_m values at a fixed level of 4.0 mM Mn^{2+} were 0.1, 0.25 and 0.29 mM for ATP, GTP and ITP, respectively. When a Mn^{2+}/nucleotide 5'-triphosphate ratio of 2 : 1 is used, these values increase to an equivalent level of 1.1 mM. The change in dissociation constant suggests that an additional effect may occur from either free Mn^{2+} or Mn-nucleotide 5'-triphosphate^{2-}.

Effect of MnATP^{2-} and Mn^{2+}. In order to investigate the roles of MnATP^{2-} and Mn^{2+} in catalysis, it was necessary to adjust the levels of these two species in the assays prior to measurement. The initial velocities recorded in Fig. 2, using fixed levels of $MnCl_2$ and variable amounts of ATP show the rate of catalysis is dependent on the concentration of MnATP^{2-}. Since the reaction proceeds at fixed levels of the metal complex in the presence of low Mn^{2+} concentrations, MnATP^{2-} must be the normal substrate. This was confirmed from the results given in Fig. 3, where assays were performed with fixed levels of ATP and variable levels of $MnCl_2$. The rate of catalysis in this instance was

TABLE IV

ABILITY OF NUCLEOTIDES TO ACT AS SUBSTRATES OF THE NON-ALLOSTERIC FORM OF PHOSPHOFRUCTOKINASE

Assays were performed in the presence of 1.0 mM fructose 6-phosphate. Relative activity values are expressed as a percentage of the activity measured with 0.2 mM ATP in the presence of Mg^{2+}. XTP, nucleotide 5'-triphosphate

Nucleotide	% Relative activity			
	Mg^{2+}/XTP ratio 10 : 1		Mn^{2+}/XTP ratio 4 : 1	
	0.2 mM	2.0 mM	0.2 mM	2.0 mM
ATP	100	181	203	427
ITP	18	67	76	121
GTP	14	52	66	103
UTP	10	25	25	61
CTP	12	34	24	55

TABLE V

APPARENT MICHAELIS-MENTEN CONSTANTS FOR VARIOUS NUCLEOSIDES OF THE NON-ALLOSTERIC FORM OF PHOSPHOFRUCTOKINASE WITH MANGANESE REPLACING MAGNESIUM AS DIVALENT METAL ION

The concentration of fructose 6-phosphate was kept at 1.0 mM. XTP, nucleotide 5'-triphosphate

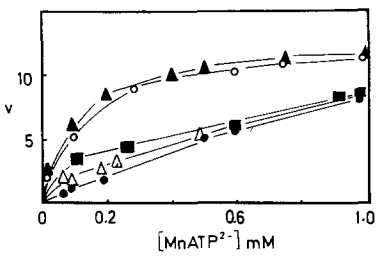
Nucleoside	V	
	Concentration ratio Mn/XTP = 2 : 1 (mM)	Concentration fixed 4.0 mM $MnCl_2$ (mM)
ATP	1.1	0.10
CTP	1.1	0.22
GTP	1.1	0.25
ITP	1.1	0.29
UTP	1.1	0.55

found to vary on $MnATP^{2-}$ levels and not on the concentration of uncomplexed Mn^{2+}.

These results cannot give conclusive evidence for the possible metal-substrate interactions at the active site. A series of fructose 6-phosphate saturation curves were performed with different concentrations of Mn^{2+} and $MnATP^{2-}$. In Table VI the calculated dissociation constants for fructose 6-phosphate show a definite decrease when free Mn^{2+} is not limiting. When the concentration of Mn^{2+} and $MnATP^{2-}$ are low (0.043 and 0.057 mM), the K_m for fructose 6-phosphate is 0.018 mM, with increasing $MnATP^{2-}$ and decreasing Mn^{2+} levels the K_m increases to 0.11 mM for 0.069 mM $MnATP^{2-}$ and 0.001 mM Mn^{2+} or to 0.12 mM for 0.49 $MnATP^{2-}$ and 0.009 mM Mn^{2+}. This trend is reversed at saturating levels of Mn^{2+} when a K_m for fructose 6-phosphate of 0.019 mM is recorded at concentrations of 0.099 mM $MnATP^{2-}$ and 3.9 mM Mn^{2+}.

Discussion

The isolation of the non-allosteric phosphofructokinase from the wild-type strain *E. coli* K-12 required the development of the described scheme, because



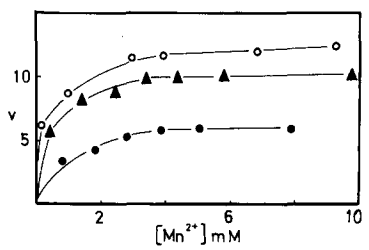


Fig. 2. Effect of $MnATP^{2-}$ on the velocity of the non-allosteric phosphofructokinase. ATP was added to solutions containing ●———●, 0.4 mM; △———△, 0.6 mM; ■———■, 1.0 mM; ○———○, 3.0 mM and ▲———▲, 4.0 mM $MnCl_2$, 1 mM fructose 6-phosphate and 0.05 M Tris-HCl buffer at pH 8.4. Assays were initiated with 2.0 μg enzyme protein.

Fig. 3. Effect of Mn^{2+} on the velocity of the non-allosteric phosphofructokinase. $MnCl_2$ was added to solutions containing ●———●, 0.1 mM; ▲———▲, 0.5 mM and ○———○, 1.0 mM ATP, 1.0 mM fructose 6-phosphate and 0.05 M Tris-HCl buffer at pH 8.4. Assays were initiated with 2.0 μg enzyme protein.

TABLE VI

EFFECT OF $MnATP^{2-}$ AND Mn^{2+} ON THE APPARENT DISSOCIATION CONSTANT FOR FRUCTOSE 6-PHOSPHATE OF THE NON-ALLOSTERIC FORM OF PHOSPHOFRUCTOKINASE FROM THE WILD-TYPE *E. coli* K-12

Fru-6-*P*, fructose 6-phosphate

$MnATP^{2-}$ (mM)	Mn^{2+} (mM)	K_mFru-6-*P* (mM)	$MnATP^{2-}$ (mM)	Mn^{2+} (mM)	K_mFru-6-*P* (mM)	$MnATP^{2-}$ (mM)	Mn^{2+} (mM)	K_mFru-6-*P* (mM)
0.043	0.057	0.018	0.049	0.45	0.024	0.049	3.95	0.018
0.068	0.032	0.019	0.098	0.40	0.022	0.099	3.90	0.019
0.088	0.002	0.090	0.43	0.07	0.061	0.498	3.50	0.029
0.069	0.001	0.110	0.49	0.009	0.120	0.997	3.0	0.026

of the initial low specific activities and the need to minimize activity losses through possible proteolytic modifications [8]. We have found that an acid-heat treatment at 50°C successfully removed more than 80% of contaminating protease without affecting enzyme activity. However, this treatment does produce an enzyme of molecular weight of 67 000. Justification for this step stems from the fact that a continual change to the low molecular weight was found to occur throughout the lengthy isolation procedure when no heat treatment was applied. Sodium dodecyl sulphate-gel electrophoresis has shown the enzyme to be a dimer of subunit molecular weight 34 000 ± 400, which is in close agreement to that found with the same protein from the mutant strain [8].

Hydroxyapatite chromatography used as an initial isolation step has been reported to produce a tetrameric enzyme of molecular weight 140 000, which binds specifically to cibracon blue-Sepharose [9]. Recent studies in our laboratory have confirmed this result and it is interesting to note that the non-sensitivity towards phospho*enol*pyruvate, non-allosteric kinetics towards fructose 6-phosphate and a lack of ADP activation are common to both the tetrameric form [9] and the dimeric form isolated in this report.

Since two pH optima were found at 6.8 and 8.4, respectively, it was of interest to investigate the kinetic properties at both pH levels. Apart from a 2-fold decrease in V at pH 6.8, there was no departure from the Michaelis-Menten kinetics found at pH 8.4 (unpublished data). A decrease in intracellular pH to acidic levels is unlikely to be of any significance to the non-allosteric protein [25] from *E. coli*.

Additional kinetics have focused attention on the ability of the protein to utilize a variety of divalent cations in complexation with the corresponding nucleotide triphosphate. In comparison, the recorded values of V with 1 mM fructose 6-phosphate for ATP in the presence of Mg^{2+} and Mn^{2+} are 5.1 and 11.3 μmol/min, respectively. The corresponding dissociation constants of 0.29 and 1.1 mM do not appear to indicate an increased affinity for the manganese complex. Yet, when $MnCl_2$ is held at a saturation level of 4 mM, the K_m for ATP is 0.1 mM (Table V) with a similar trend occurring for the other nucleosides.

A study on the effect on the rate of catalysis for a wide range of calculated values of $MnATP^{2-}$ and uncomplexed Mn^{2+} shows conclusively, that there is a

definite concentration dependence for the true substrate $MnATP^{2-}$ rather than a catalytic effect from free Mn^{2+}. However, a survey of the range of dissociation constants obtained with calculated levels of $MnATP^{2-}$ and free Mn^{2+} (Table VI) shows that an effect on the substrate affinity for fructose 6-phosphate does occur in the case of the uncomplexed Mn^{2+}. Provided Mn^{2+} is not limiting, the K_m for fructose 6-phosphate remains relatively constant at 0.018 mM.

An interpretation of this data would indicate that complexed manganese as $MnATP^{2-}$ is a prerequisite for enhanced catalysis, and by comparison, a more efficient substrate than $MgATP^{2-}$. Free Mn^{2+} on the other hand does change the affinity for the substrate fructose 6-phosphate without affecting V. Although it has been assumed that the formation of a metal-fructose 6-phosphate complex is negligible [26], a compound of this nature could affect the increase in affinity for this substrate. Alternatively, an enzyme metal interaction could produce a similar result. Under the conditions of these experiments it is not possible to conclude a binding of free Mn^{2+} to the protein without an accurate measurement of possible enzyme-Mn^{2+} ternary complexes. In rabbit muscle, by means of magnetic resonance studies [27], it was shown, that when Mn^{2+} replaced Mg^{2+}, the divalent cation may be directly bound to the phosphofructokinase protein. A recent study by Hofmann's group [28] utilizing equilibrium dialysis provides direct evidence that Mn^{2+} does have three independent binding sites per enzyme subunit of yeast phosphofructokinase. However, the question still remains, as to whether these binding sites could be coordinated to nucleotide substrate and activator site, respectively. As suggested by Jones et al. [29] the stability of $MnATP^{2-}$ complexes probably do suggest a metal-nucleotide complex formation prior to enzyme interaction with the metal. The same enzyme from brain has been reported to utilize both $MnATP^{2-}$ and $CoATP^{2-}$ as substrates [30] and $MnATP^{2-}$ was also observed to activate this enzyme from yeast [28].

No known metabolite effectors have yet been discovered, which indicates a negative metabolic control function for the non-allosteric enzyme. However, the enzyme exhibits little discrimination between nucleotide triphosphate substrates in contrast to the allosteric isozyme [31]. It is interesting to note that a recent growth/yield study [6] using isogenic strains of *E. coli* has placed some doubt on the importance of the allosteric nature of phosphofructokinase in glycolysis. Therefore, an enzyme unaffected by phospho*enol*pyruvate must provide *E. coli* with a means of maintaining some flow of carbon when conditions are suboptimal for normal phosphofructokinase activity.

Acknowledgement

This work has been carried out with the financial help of the Australian Research Grant Commission, University Research Grant and the Australian Postgraduate Award Scheme (K.N.E.), for which we are extremely grateful.

References

1 Doelle, H.W. (1972) Biochim. Biophys. Acta 258, 404—410
2 Simon, W.A. and Hofer, H.W. (1977) Biochim. Biophys. Acta 481, 450—462

3 Ferdinandus, J. and Clark, J.B. (1969) Biochem. J. 103, 735—736
4 Doelle, H.W. (1972) Proc. Aust. Biochem. Soc. 5, 30
5 Fraenkel, D.G., Kotlarz, D. and Buc, H. (1973) J. Biol. Chem. 248, 4865—4866
6 Robinson, J.P. and Fraenkel, D.G. (1978) Biochem. Biophys. Res. Commun. 81, 858—863
7 Kotlarz, D., Garreau, H. and Buc, H. (1975) Biochim. Biophys. Acta 381, 257—268
8 Kotlarz, D. and Buc, H. (1977) Biochim. Biophys. Acta 484, 35—48
9 Babul, J. (1978) J. Biol. Chem. 253, 4350—4355
10 Thomas, A.D., Doelle, H.W., Westwood, A.W. and Gordon, G.L. (1972) J. Bacteriol. 112, 1099—1105
11 Ewings, K.N. and Doelle, H.W. (1976) Eur. J. Biochem. 69, 563—570
12 Reichelt, J.L. and Doelle, H.W. (1971) Antonie van Leeuwenhoek J. Microbiol. Serol. 37, 497—506
13 Doelle, H.W. (1978) Eur. J. Biochem. 83, 479—484
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
15 Vinuela, E., Salas, M.L. and Sols, A. (1963) Biochem. Biophys. Res. Commun. 12, 140—145
16 O'Sullivan, W.J. and Perrin, D.D. (1964) Biochemistry 3, 18—26
17 Akkerman, J.W.N., Gorter, G., Sixma, J.J. and Gerhard, E.J. (1974) Biochim. Biophys. Acta 370, 113—119
18 O'Sullivan, W.J. and Noda, M. (1968) J. Biol. Chem. 243, 1424—1433
19 Ahlers, J. (1977) Mol. Cell. Biochem. 15, 145—149
20 Segel, I.H. (1976) Enzyme Kinetics, J. Wiley and Sons, New York
21 Andrews, P. (1964) Biochem. J. 91, 222—233
22 Martin, R.G. and Ames, B.N. (1961) J. Biol. Chem. 263, 1372—1379
23 Weber, K. and Osborne, M. (1969) J. Biol. Chem. 244, 4406—4412
24 Vesterberg, O., Hansen, L. and Sjosten, A. (1977) Biochim. Biophys. Acta 491, 160—166
25 Trivedi, B. and Danforth, W.H. (1966) J. Biol. Chem. 241, 4110—4112
26 Schwarzenbach, G. and Anderegg, G. (1957) Helv. Chim. Acta 40, 1227
27 Cottam, G.L. and Uyeda, K. (1973) Arch. Biochem. Biophys. 154, 683—690
28 Peters, W.H., Nissler, K., Schellenberger, W. and Hofmann, E. (1979) Biochem. Biophys. Res. Commun. 90, 561—566
29 Jones, R., Dwek, R.A. and Walker, I.O. (1972) Eur. J. Biochem. 28, 74—82
30 Muntz, J.A. (1953) Arch. Biochem. Biophys. 42, 435—445
31 Blangy, D., Buc, H. and Monod, J. (1968) J. Mol. Biol. 31, 13—35