The effects of magnesium ions on the interactions of ox brain and liver glutamate dehydrogenase with ATP and GTP

Alun D. McCARTHY* and Keith F. TIPTON
Department of Biochemistry, Trinity College, Dublin 2, Ireland

(Received 10 February 1984/Accepted 17 April 1984)

The effects of ATP and GTP on the activities of ox liver and brain glutamate dehydrogenase were determined in the absence and presence of added Mg^{2+} ions. Although GTP was an inhibitor of the enzyme reaction assayed in the direction of NAD^+ reduction, the magnesium complex of this nucleotide had no effect on the activity. Similarly the magnesium complex of ATP was without effect on the activity of the enzyme although the free nucleotide was an activator. These results suggest that it is important to take account of magnesium complex formation when considering the regulatory actions of these nucleotides.

The activity of the enzyme glutamate dehydrogenase [L-glutamate:NAD(P)⁺ oxidoreductase (deaminating), EC 1.4.1.3] has been shown to be affected by nucleoside di- and tri-phosphates. GTP and GDP have been shown to be allosteric inhibitors of the enzyme under most conditions, whereas ATP and ADP are activators (for reviews see Smith *et al.*, 1975; Fisher, 1973). The effects of these compounds depend, however, on the nature of the coenzyme used and the assay pH (Di Prisco, 1975; Bailey *et al.*, 1982).

Although many studies have been carried out on the regulation of glutamate dehydrogenase activity by nucleoside triphosphates, and it has been suggested that they play an important role in modulating its activity (see, e.g., Frieden, 1968), these studies have used the free, uncomplexed, ligand. However, in the mitochondrion it is likely that a proportion of these nucleotides will exist as metal-ion complexes. Since the allosteric effects of ATP and ADP on other enzymes have been shown to be altered by metal-ion chelation (Fromm, 1979) we have studied the effects of Mg^{2+} ions on the regulation of the activity of purified ox brain and liver glutamate dehydrogenase by ATP and GTP. Commercially obtained preparations of the ox liver enzyme have been shown to have suffered limited proteolysis but preparations of the ox liver and brain enzymes that had not suffered such proteolysis have been found to be identical in a number of physical and protein-chemical properties (McCarthy *et al.*, 1980, 1981). Thus this study

also allowed a comparison of the sensitivities of proteolysed and unproteolysed preparations of the enzyme to the effects of these nucleoside triphosphates.

Materials and methods

Ox brain glutamate dehydrogenase was purified as described by McCarthy *et al.* (1980) and the enzyme from ox liver was obtained, as a suspension in 50% glycerol, from Boehringer Corp. (London) Ltd.

Enzyme activity in the direction of oxidative deamination was determined fluorimetrically at 30°C by following the rate of reduction of NAD^+ in a reaction mixture containing 1 mM- NAD^+ , 40 mM-L-glutamate, 50 mM-sodium/potassium phosphate buffer, pH 7.4, enzyme (0.12 µg/ml) and, where appropriate, 20 mM- $MgSO_4$ and/or GTP or ATP. $MgSO_4$ at this concentration was found to have no effect on the activity of either enzyme preparation assayed in the absence of nucleoside phosphates. Similar concentrations of magnesium acetate were, however, found to be inhibitory. Initial velocity was shown to be a linear function of enzyme concentration with both preparations in the concentration range of 0.02–0.4 µg/ml in the assay mixture.

Since several of the components of the reaction mixture can form complexes with Mg^{2+} it was necessary to calculate the free concentration of these ions. Duggleby & Dennis (1970) have reported a value of 10 mM for the Mg - NAD complex, which is sufficiently high for the effect of

* Present address: Glaxo Group Research, Greenford Road, Greenford, Middlesex UB6 0HE, U.K.

the formation of this complex to be negligible under the conditions used in this work. In the absence of added nucleoside triphosphate, the equations shown in Scheme 1 describe the binding of Mg^{2+} to the components of the assay system. Values for the dissociation constants for the complexes of Mg^{2+} with glutamate and HPO_4^{2-} , at 25–30°C and 10.01–0.5, were taken as 12.6mM and 3.2mM respectively (Dawson *et al.*, 1969). From these values and the equations shown in Scheme 1, the free concentration of Mg^{2+} ions in the assay mixture was calculated to be 1.5mM.

The dissociation constants for the Mg^{2+} complexes with ATP and GTP were determined at 30°C in 100mM-triethanolamine/HCl buffer, pH 7.4, containing 100mM- NaNO_3 and 0.42mM-8-hydroxyquinoline by the method of Burton (1959). Concentrations of nucleoside triphosphates in the range 0.7–3.0mM were used and the dissociation constants were obtained by extrapolating the observed values to a nucleotide concentration of zero.

In the presence of GTP and ATP a more complicated scheme than that shown in Scheme 1 would be required fully to describe the system. However, the relatively low concentrations of these nucleotides that were used in these studies (up to 2mM-ATP or 0.4mM-GTP) would not have

any significant effect on the degree of saturation of the enzyme with glutamate, and the effects on the free magnesium concentration in the assay mixture would be minimized because the excess magnesium bound to phosphate and glutamate would act as magnesium-ion buffers (Morrison, 1979).

Results and discussion

Dissociation constants of 73 μM and 100 μM were determined for the magnesium complexes with ATP and GTP respectively. The former value is consistent with that reported for MgATP^{2-} and MgHATP^- by Morrison (1979). These values indicate that, in the presence of 1.5mM free Mg^{2+} , no more than 5% of the ATP or 6.3% of the GTP would be present in the uncomplexed form at the concentrations used in these studies.

In the absence of added Mg^{2+} , ATP was an activator of ox liver and brain glutamate dehydrogenase with apparent K_a values of 130 μM and 85 μM respectively. The maximum extent of activation (approx. 2.6-fold), determined from linear graphs of the reciprocal of the increase in velocity versus the reciprocal ATP concentration, was, however, the same for both preparations. In the presence of Mg^{2+} , however, no effect of ATP on initial rate could be observed at concentrations of up to 2mM. If the complex between Mg^{2+} and ATP had no effect on the enzyme it may be calculated that, at a free Mg^{2+} concentration of 1.5mM, the observed K_a values due to the free nucleotide remaining in solution would be about 2.6mM and 1.8mM for the liver and brain enzymes respectively. Thus, although the MgATP complex is neither an activator nor an inhibitor of glutamate dehydrogenase under these conditions, the lack of any observed effect might suggest that this complex could bind to the enzyme and compete with the free ATP.

In the absence of added Mg^{2+} , GTP was an inhibitor of the ox brain and liver enzymes with apparent K_i values of 6.5 μM and 9.5 μM respectively. If only the uncomplexed form of this nucleotide were to affect the enzyme, these values would rise to about 104 μM and 140 μM , respectively, in the presence of 1.5mM free Mg^{2+} . The results shown in Fig. 1 show that under these conditions the K_i values determined for the brain and liver enzymes were 115 μM and 200 μM respectively. Thus it appears that, within experimental error, the uncomplexed GTP in solution could account for the observed inhibition and the MgGTP complex has no significant effect under these conditions.

In contrast, Bailey *et al.* (1982) have stated that Mg^{2+} has no effect on the activation of glutamate dehydrogenase by ADP. The effects of complex formation with ADP could not be studied adequately in the present system, however, because

Equations:

$$K_a = \frac{[\text{H}^+][\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$$

$$K_p = \frac{[\text{Mg}_f^{2+}][\text{HPO}_4^{2-}]}{[\text{Mg}\cdot\text{HPO}_4]}$$

$$K_g = \frac{[\text{Mg}_f^{2+}][\text{Glutamate}_f]}{[\text{Mg}\cdot\text{glutamate}]}$$

$$\text{Mg}^{2+}_{\text{total}} = \text{Mg}_f^{2+} + \text{Mg}\cdot\text{HPO}_4 + \text{Mg}\cdot\text{glutamate}$$

$$\text{Phosphate}_{\text{total}} = \text{H}_2\text{PO}_4^- + \text{HPO}_4^{2-} + \text{Mg}\cdot\text{HPO}_4$$

$$\text{Glutamate}_{\text{total}} = \text{Glutamate}_f + \text{Mg}\cdot\text{glutamate}$$

Scheme 1. Binding of Mg^{2+} ions to components of the glutamate dehydrogenase assay

The subscript 'f' denotes free concentrations.

Fig. 1. Inhibition of ox liver (○) and brain (●) glutamate dehydrogenase activity by GTP in the presence of 20 mM- Mg^{2+}

The activities were determined in the direction of oxidative deamination of glutamate with NAD^+ as the coenzyme. Experimental details are given in the text.

the considerably lower affinity of this nucleotide for Mg^{2+} (Dawson *et al.* 1969; Morrison, 1979) would necessitate the presence of a higher Mg^{2+} concentration in the assay mixture to ensure a high degree of complex formation, and this would, in turn, significantly deplete the free substrate concentration.

It is difficult to estimate the free concentrations of Mg^{2+} in mitochondria, but Kohn *et al.* (1979) have reported values of 0.1 mM in resting dog heart mitochondria which rise to about 1 mM during exercise. Such changes, regardless of any concomitant changes in the total concentrations of nucleoside phosphates, would have a profound influence on the abilities of GTP and ATP to affect the activity of glutamate dehydrogenase. In contrast,

any effects on the free ADP and GDP concentrations would be expected to be considerably smaller because of the considerably higher dissociation constant of the MgADP complex (Dawson *et al.* 1969; Morrison, 1979). Thus any evaluation of the effects of ATP and GTP on the activity of glutamate dehydrogenase must take account of the effects of complex formation with Mg^{2+} reducing their apparent potencies.

The proteolysed, ox liver, preparation of glutamate dehydrogenase was found to be rather less sensitive to inhibition by GTP and activation by ATP than the unproteolysed, ox brain, enzyme. The former effect may be related to the greater sensitivity of unproteolysed preparations to depolymerization by GTP and NADH at higher enzyme concentrations (McCarthy *et al.*, 1981). However, assessment of the significance of these effects in terms of the structures of the nucleotide binding sites and the behaviour of the enzyme *in vivo* must await the results of further studies.

We are grateful to the Trinity College Development Fund for a grant in support of this work.

References

- Bailey, J., Bell, E. T. & Bell, J. E. (1982) *J. Biol. Chem.* **257**, 5579–5583
- Burton, K. (1959) *Biochem. J.* **71**, 388–395
- Dawson, R. M. C., Elliott, D., Elliott, W. H. & Jones, K. M. (1969) *Data for Biochemical Research*, pp. 423–434, Clarendon Press, Oxford
- Di Prisco, A. (1975) *Arch. Biochem. Biophys.* **171**, 604–612
- Duggleby, R. G. & Dennis, D. T. (1970) *J. Biol. Chem.* **245**, 3745–3750
- Fisher, H. F. (1973) *Adv. Enzymol.* **39**, 369–418
- Frieden, C. (1968) in *The Role of Nucleotides for the Function and Conformation of Enzymes* (Kalckar, H. M. *et al.*, eds.), pp. 194–225, Munksgaard, Copenhagen
- Fromm, H. J. (1979) *Trends Biochem. Sci.* **2**, N198–N200
- Kohn, M. C., Achs, M. J. & Garfinkel, D. (1979) *Am. J. Physiol.* **237**, R159–R166
- McCarthy, A. D., Walker, J. M. & Tipton, K. F. (1980) *Biochem. J.* **191**, 605–611
- McCarthy, A. D., Johnson, P. & Tipton, K. F. (1981) *Biochem. J.* **199**, 235–238
- Morrison, J. F. (1979) *Methods Enzymol.* **63**, 257–294
- Smith, E. L., Austen, B. M., Blumenthal, K. M. & Nyc, J. F. (1975) *Enzymes 3rd Ed.* **11**, 293–367