THE DEPENDENCE OF PHOSPHOFRUCTOKINASE KINETICS UPON PROTEIN CONCENTRATION

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Received 12 November 1970

1. Introduction

It is well established that mammalian phosphofructokinases show concentration-dependent association and dissociation phenomena [1–5]. The minimum fully active form of the enzyme from skeletal muscle has a molecular weight of 380,000 and can be dissociciated into units of molecular weight 192,000 by dilution at pH 6.7, or lowering the pH to 5.0 [1]. The dissociated enzyme has at most two percent of the activity of the associated form. Similar observations have been made on the enzyme from sheep heart [2]. In the latter case ATP was found to favour dissociation, and the other substrate of the reaction, fructose-6-phosphate (F6P) exerted a countervailing effect, tending to favour the associated form of the enzyme.

The results presented here indicate that the effector modulated dissociation of phosphofructokinase is capable of accounting for almost the entire range of control properties of the enzyme. These may be summarised as (1) inhibition by high concentrations of ATP, (2) synergistic inhibition by citrate in the presence of inhibitory concentrations of ATP, (3) the counteraction of inhibition by adenosine monophosphate (AMP) and fructose-1-6-diphosphate (FDP), and (4) cooperativity of the F6P kinetics in the presence of inhibitors. In relation to (3) it should be noted that although the kinetic order of AMP activation appears to be two, whilst that of FDP activation is one, both compounds are capable of completely counteracting ATP inhibition.

2. Experimental

Phosphofructokinase was prepared from beef heart by the method of Frenkel [6]. The preparation used in these studies had a specific activity of 46 international units per milligram protein, measured at pH 8.0 and 25°, which is rather less than the specific activites of between 75 and 100 which the technique has usually yielded in our hands. However the kinetic parameters of the enzyme were identical to those measured using more highly purified preparations, the Michaelis constants for ATP and F6P measured in the presence of saturating quantities of AMP being 42 and 112 μM, respectively. The response of the enzyme to effectors also differed in no respect from the behaviour observed previously with a number of different enzyme preparations.

Phosphofructokinase was assayed at pH 6.70 and 25° using a Radiometer 26 pH meter coupled to a vitatron recorder at a sensitivity of 0.1 pH units full scale, to monitor the release of protons concommitant upon the progress of the reaction. The reaction buffer contained 100 mM potassium chloride, 10 mM magnesium sulphate, and 1 mM imidazole chloride, Initial rates of reaction were measured at a pH not more than 0.02 units below 6.70, and corrected for the buffering capacities of the substrates and effectors present in the reaction mixture (which were determined under the conditions of the experiment) using an A.S.A. Fortran programme written for the University of Cambridge Titan computer. Reactions were initiated by the addition of the enzyme as a sludge suspended in a solution containing 10 mM potassium phosphate, and 60 percent w/v ammonium sulphate, pH 7.0.

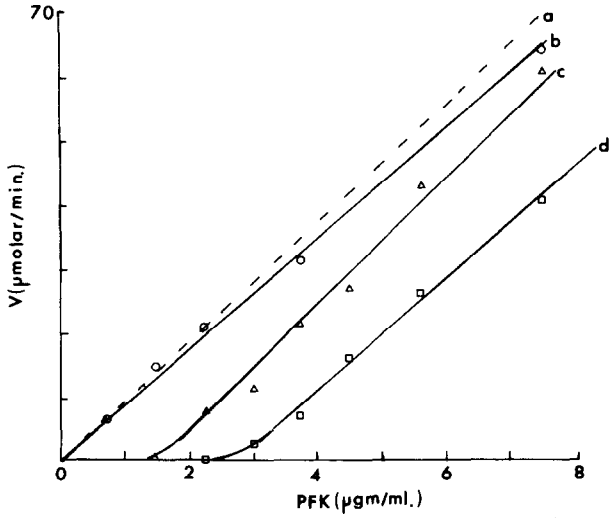

Fig. 1. (a) Theoretical plot for F6P = 200 μM, ATP = 500 μM, at saturating AMP; (b) F6P = 200 μM, ATP = 200 μM; AMP = 200 μM; (c) F6P = 200 μM, ATP = 500 μM; (d) F6P = 200 μM, ATP = 500 μM, citrate = 500 μM.

Controls were carried out by the addition of the supernatant from a centrifuged solution of the enzyme, which had no effect on the observed rates of reaction.

Fig. 1 shows that the dependence of rate on enzyme concentration, although linear in the presence of a saturating concentration of AMP is non linear in the pres-

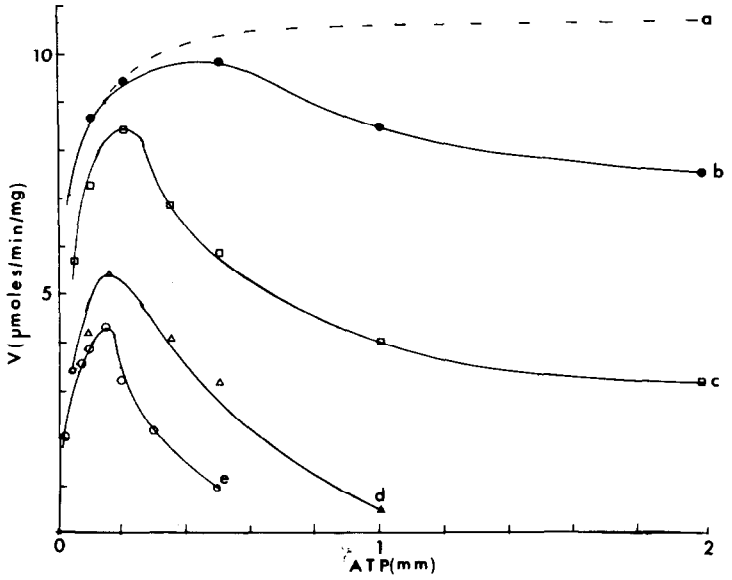

Fig. 2. Plots of normalised rate against ATP concentration at 200 μM F6P and a series of different enzyme concentrations. (a) Expected curve at saturating AMP; (b) Enzyme concentration = 7.5 μg/ml; (c) Enzyme concentration = 3.75 μg/ml; (d) Enzyme concentration = 2.6 μg/ml; (e) Enzyme concentration = 1.87 μg/ml.

ence of a high concentration of ATP, at low protein concentrations. Citrate exacerbates this non-linearity. However, at sufficiently high enzyme concentrations, the curves become linear, and the relative degree of inhibition decreases. The initial non-linearity strongly suggests that the enzyme is dissociating into an inactive form at low protein concentrations, and that inhibitors increase this effect, presumably by binding to the dissociated form. Activators prevent dissociation, presumably by binding to the associated form of the enzyme. The process of reassociation appears to be fast, since addition of AMP, or an additional aliquot of enzyme to an inhibited sample causes an immediate activation. The amount of dissociated enzyme present will become proportional to the square root of the total enzyme concentration at high values of the latter, and so the ratio of dissociated to associated forms will fall to zero as the enzyme concentration rises toward infinity.

The striking dependence of inhibition by high concentrations of ATP on protein concentration is shown in fig. 2. Evidently, as the enzyme concentration rises, the rate against ATP profile approaches more closely to the hyperbola which would be observed in the presence of saturating concentrations of AMP, and the extremum of the curve moves toward progressively higher ATP concentrations. There is a marked correspondence between these rate profiles and those obtained by varying ATP concentrations at a series of F6P concentrations greater than the K_m for F6P, which seems to simulate the effect of increasing the enzyme concentration. F6P must thus bind to the catalytic site of the enzyme only when it is in its associated form. Studies on the isotope exchange reactions of phosphofructokinase are consistent with this, and also suggest that ATP can bind to the catalytic site in both the associated and dissociated forms [13]. Thus ATP binding at the catalytic site would not be expected to have the same effect as binding of F6P.

3. Discussion

The model of phosphofruktokinase proposed here subsumes a considerable amount of information. It not only provides a rational basis for consideration of the kinetics of enzyme but is capable of reconciling the cooperativity of this behaviour with respect to ATP and F6P with the non-cooperative nature of the binding kinetics [7, 8]. It also explains the activating effect of AMP at apparently non-inhibitory ATP concentrations, and the extreme synergism of ATP and citrate inhibition, which is hard to account for on any other basis. The results of ultracentrifugation and sucrose density gradient studies showing rapid association and dissociation [1–5] are a necessary consequence of the model. Similar behaviour has been observed in a number of other cases, the best known of which is glutamate dehydrogenase [8], and may be a more important source of control mechanisms than has previously been realised.

The metabolic consequences of the control mechanism for phosphofruktokinase are interesting. The activity of the enzyme in rat heart has been estimated to be about 20 international units per gram wet weight [10], corresponding to about 40 units per ml of cytoplasm. This concentration is about 120 times the maximum used in these kinetic studies, but ATP levels have been estimated to be of the order of 10 mM in rat heart [11, 12], which is only about 5 times the maximum concentration used in this work. This seems to imply that ATP alone is likely to be a rather poor inhibitor of phosphofruktokinase in vivo. Citrate too is rather a poor inhibitor per se, and probably ATP and citrate levels have to be simultaneously high to cause inhibition of the enzyme unless compartmentation is responsible for locally exaggerated ATP concentrations.

Acknowledgements

E.C.H. wishes to thank the Science Research Council for a research studentship, during the tenure of which this work was performed.

References

[1] V. Paetkau and H.A. Lardy, J. Biol. Chem. 242 (1967) 2035
[2] T.E. Mansour and C.E. Ahlfors, J. Biol. Chem. (1968) 2523.
[3] T.E. Mansour, N. Wakid and H.M. Sprouse, J. Biol. Chem. 241 (1966) 1512.
[4] A. Parmeggiani et al., J. Biol. Chem. 241 (1966) 4625.
[5] K.-H. Ling, F. Marcus and H.A. Lardy, J. Biol. Chem. 240 (1965) 1894.

[6] R. Frenkel, Arch. Biochem. Biophys. 125 (1968) 166.
[7] R.G. Kemp and E.G. Krebs, Biochemistry 6 (1967) 423.
[8] M.Y. Lorenson and T.E. Mansour, J. Biol. Chem. 244 (1969) 6420.
[9] C. Frieden and R.F. Colman, J. Biol. Chem. 242 (1967) 1705.
[10] M.C. Scrutton and M.F. Utter, Ann. Rev. Biochem. 37 (1968) 669.
[11] R.A. Denton et al., Biochem. J. 100 (1968) 407.
[12] E.A. Newsholme and P.J. Randle, Biochem. J. 93 (1964) 641.
[13] E.C. Hulme and K.F. Tipton, (1970) Biochem. J., submitted.