The binding of glucose to yeast hexokinase monomers is independent of ionic strength

Elaine L. MAYES, James G. HOGGETT and George L. KELLETT
Department of Biology, University of York, Heslington, York YO1 5DD, U.K.

(Received 1 February 1982/Accepted 15 February 1982)

Hoggett & Kellett [*Eur. J. Biochem.* **66**, 65–77 (1976)] have reported that the binding of glucose to the monomer of hexokinase PII isoenzyme is independent of ionic strength, in contrast to the subsequent claim of Feldman & Kramp [*Biochemistry* **17**, 1541–1547 (1978)] that the binding is strongly dependent on ionic strength. Since measurements with native hexokinase P forms are complicated by the fact that the enzyme exists in a monomer–dimer association–dissociation equilibrium, we have now studied the binding of glucose to the proteolytically-modified S forms which are monomeric. At pH 8.5, the affinity of glucose for both SI and SII monomers is independent of salt concentration over the range of KCl concentrations 0–1.0 mol·dm⁻³ and is in good agreement with that of the corresponding P forms in both low and high salt. These observations confirm that the binding of glucose to hexokinase P monomers is independent of ionic strength and that the affinity of glucose for the hexokinase PII monomer is about an order of magnitude greater than that for the dimer.

Yeast hexokinase PI and PII isoenzymes exist as dimers of a subunit of M_r 50 000 (Schulze & Colowick, 1969; Easterby & Rosemeyer, 1972; Derechin *et al.*, 1972; Schmidt & Colowick, 1973; Hoggett & Kellett, 1976a). The three-dimensional structure of the subunit comprises two lobes separated by a deep glucose-binding cleft (Bennett & Steitz, 1978). Binding of glucose induces a gross conformational change in which one lobe rotates relative to the other by 12° and results in strong enhancement of nucleotide binding (Colowick *et al.*, 1969; De La Fuente *et al.*, 1970). It is therefore of interest to study the binding of glucose to hexokinase.

However, binding studies are complicated by the fact that both yeast isoenzymes also exist in an association–dissociation equilibrium between monomer and dimer that is dependent on several factors, including pH, ionic strength and glucose concentration (Colowick *et al.*, 1969; Schulze & Colowick, 1969; Easterby & Rosemeyer, 1972; Derechin *et al.*, 1972; Hoggett & Kellett, 1976a). Glucose causes dissociation of dimer to monomer, implying that monomer has a greater affinity for glucose than does dimer. In a previous study on the binding of glucose to yeast hexokinase PII isoenzyme and its influence on the monomer–dimer association–dissociation equilibrium we concluded that the affinity of the monomer for glucose is approx. 20-, 8- and 5-fold that of the dimer at pH values of 6.7,

7.0 and 8.0 respectively (Hoggett & Kellett, 1976a). The affinity of the monomer was found to be independent of ionic strength over the range of KCl concentrations 0–1.0 mol·dm⁻³ at low and high pH; the large (up to about 8-fold at neutrality) increases in the affinity of the enzyme observed with increases in ionic strength under these conditions, where the enzyme exists as a mixture of monomers and dimers, were fully accounted for by increases in the dissociation of dimer to monomer. Subsequently, Feldman & Kramp (1978) have criticized these conclusions, claiming that at pH 8.3 the affinity of glucose for the monomer is only about 1.6- to 2.0-fold greater than that for the dimer and attributing most of the dependence of glucose binding to the enzyme on ionic strength to a 4-fold decrease in the intrinsic dissociation constant of glucose from the monomer with increase in ionic strength.

The ideal course to resolve this difference of view would be to study the binding of glucose to either monomer or dimer alone. However, this is not practicable under all except a very few conditions with native yeast hexokinase. In practice, detailed knowledge of both the association–dissociation equilibrium as a function of glucose concentration and the binding of glucose is required. Thus there are two potential sources of error, and conclusions about the intrinsic affinity of the monomer for glucose often have to be based on linkage calculations.

For these reasons we describe here an alternative

approach to this question by making use of the proteolytically-modified S forms of yeast hexokinase. Both the PI and PII isoenzymes may be converted, by cleavage of an 11-residue peptide from the *N*-terminus, to the proteolytically-modified forms SI and SII (Schmidt & Colowick, 1973). The terminal peptide is intimately involved in the self-association of subunits and it is well-established that the S forms, which lack the peptide, are monomeric under all normal experimental conditions above pH 6 whether at low or at high ionic strength (Colowick *et al.*, 1969; Easterby & Rosemeyer, 1972; Schmidt & Colowick, 1973). Moreover, the S forms have been reported by Colowick *et al.*, (1979) to be good analogues of the monomeric P forms. The S forms thus offer the advantage that the binding of glucose to monomers alone can be investigated without any complications arising from monomer-dimer association-dissociation.

Experimental

Yeast hexokinase PI and PII isoenzymes were prepared as described by Hoggett & Kellett (1976a). The isoenzymes were then converted to the corresponding S forms and purified as described by Schmidt & Colowick (1973). Both SI and SII were homogeneous as judged by polyacrylamide-gel electrophoresis. All reagents were of AnalaR grade.

The binding of glucose to hexokinase was monitored by the concomitant quenching of protein fluorescence as described previously (Hoggett & Kellett, 1976a). Enzyme was excited at 285 nm and fluorescence was observed at 325 nm.

Results and discussion

Representative titrations of the quenching of protein fluorescence as a function of glucose concentration in the presence and absence of KCl ($1.0 \text{ mol} \cdot \text{dm}^{-3}$) at pH 8.5 in Tris/HCl buffer ($50 \text{ mmol} \cdot \text{dm}^{-3}$) are presented in Fig. 1(a) for hexokinase SI and in Fig. 1(b) for hexokinase SII. Hill plots of these and all other titrations reported here are linear with Hill coefficients in the range 0.95–1.05. As expected then for a molecule possessing only one site, binding of glucose is non-co-operative and changes in fluorescence are directly proportional to changes in binding.

The titration curves were therefore analysed according to the hyperbolic function:

$$F_G = F_0 + \Delta F_{\max} \left(\frac{[G]}{K + [G]} \right)$$

in which F_G is the fluorescence at a given concentration of glucose, $[G]$, F_0 is that at zero concentration, $\Delta F_{\max}$ is the maximal quenching of fluorescence and K is the dissociation constant of glucose.

Fig. 1. Dependence of the quenching of the intrinsic protein fluorescence of yeast hexokinase SI and SII monomers as a function of glucose concentration in Tris/HCl buffer ($50 \text{ mmol} \cdot \text{dm}^{-3}$, pH 8.5) containing either no KCl (□) or KCl at $1.0 \text{ mol} \cdot \text{dm}^{-3}$ (○)

Enzyme concentration $0.05 \text{ g} \cdot \text{dm}^{-3}$; temperature 20.0°C . (a), Hexokinase SI; (b), hexokinase SII.

$\Delta F_{\max}$ and K were computed for each titration curve by standard non-linear least-squares procedures.

$\Delta F_{\max}$ was found to be independent of ionic strength for forms SI and SII and to have average values of 12% and 16% respectively relative to F_0 . The dissociation constants of glucose from hexokinase forms SI and SII at KCl concentrations between 0 and $1.0 \text{ mol} \cdot \text{dm}^{-3}$ at pH 8.5 are presented in Table 1. It is apparent that the binding of glucose to either form SI or form SII shows no dependence on ionic strength, the average values of their glucose dissociation constants over this range being 0.22 ± 0.02 and $0.03 \pm 0.02 \text{ mmol} \cdot \text{dm}^{-3}$ respectively. This finding is in good agreement with our previous conclusion that the binding of glucose to hexokinase PII monomers is independent of ionic strength (Hoggett & Kellett, 1976a). This conclusion is strengthened all the more by the fact that, under the present experimental conditions, the S forms are entirely monomeric (Colowick *et al.*, 1969; Easterby & Rosemeyer, 1972; Schmidt & Colowick, 1973); the binding experiments with S forms are therefore not

Table 1. Dissociation constants of glucose from yeast hexokinase SI and SII monomers as a function of KCl concentration

[KCl] (mol·dm ⁻³)	K (mmol·dm ⁻³) for form:	
	SI	SII
0	0.20	0.32
0.1	0.20	0.31
0.2	0.23	0.28
0.5	0.23	0.31
1.0	0.23	0.30
Mean ± S.D.	0.22 ± 0.02	0.30 ± 0.02

complicated by the presence of dimer and are technically straightforward.

The present experiments were carried out under conditions very similar to those of Feldman & Kramp (1978) and to the original experiments of Hoggett & Kellett (1976a) at pH 8.0 with native P forms. It is not clear why their results differed. However, it should be noted that the experiments of Feldman and Kramp were performed only at a single enzyme concentration for a given isoenzyme. Failure to study a range of concentrations, so that the proportions of monomer and dimer in a mixture are varied, makes it extremely difficult to resolve their individual properties. It was because of the need to distinguish accurately between monomer and dimer that the fluorescence titrations done by Hoggett & Kellett (1976a) were performed over an enzyme concentration range of 100- to 1000-fold under each condition.

The question arises as to how far the proteolytically-modified S forms are suitable analogues of the native P forms. Colowick *et al.* (1969) have long argued that they are good analogues on the basis of direct binding studies and of the catalytic identity of S forms and the corresponding P forms at very low concentrations in steady-state assays.

The binding data of Hoggett & Kellett (1976a) support their conclusions. Thus the dissociation constants of glucose from hexokinase PI and PII monomers produced at pH 8 in KCl (1.0 mol·dm⁻³) have been determined as 0.12 mmol·dm⁻³ (Hoggett & Kellett, 1976b) or 0.19 mmol·dm⁻³ (E. L. Mayes, J. G. Hoggett & G. L. Kellett, unpublished work) for PI and as 0.30 mmol·dm⁻³ for PII (Hoggett & Kellett, 1976a,b). These values compare with those of 0.22 and 0.30 mmol·dm⁻³ for forms SI and SII respectively (Table 1). In addition, the dissociation constant of glucose from hexokinase PII in Tris/HCl buffer (20 mmol·dm⁻³, pH 8) in the absence

of KCl was found to be 0.25 ± 0.05 mmol·dm⁻³ at a concentration of 1 mg·dm⁻³ (Fig. 6d of Hoggett & Kellett, 1976a). Under these conditions the enzyme exists as more than 97% monomer throughout the whole fluorescence titration. The same argument and conclusions apply also to data at pH 7.0 and KCl (0.1 mol·dm⁻³) (Fig. 7a of Hoggett & Kellett, 1976a). Thus there is direct, albeit limited, experimental evidence that the binding of glucose to native PII monomers is independent of ionic strength.

The data above therefore confirm the conclusions of Hoggett & Kellett (1976a) that the affinity of glucose for hexokinase PII monomer is about 20-, 8- and 5-fold that of the dimer at pH values of 6.7, 7.0 and 8.0 respectively, rather than the factor of 2 claimed by Feldman & Kramp (1978). The situation for PI is more complicated than that for PII. Whereas the PII dimer binds glucose non-cooperatively at all pH values, the PI dimer binds glucose co-operatively at low pH values and non-cooperatively at high pH values (Tickner *et al.*, 1976; Hoggett *et al.*, 1977). However, in work to be described in detail elsewhere, we have determined that the ratio (affinity of glucose for PI monomer : affinity of glucose for PI dimer) is independent of pH above pH 7 and has a value of about 6.

E. L. M. was the recipient of a Science Research Council studentship.

References

- Bennett, W. S., Jr., & Steitz, T. A. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4848–4852
- Colowick, S. P., Womack, F. C. & Nielson, J. (1969) in *The Role of Nucleotides for the Function and Conformation of Enzymes* (Kalckar, H. M., ed.), pp. 15–43, Munksgaard, Copenhagen
- De La Fuente, G., Lagunas, R. & Sols, A. (1970) *Eur. J. Biochem.* **16**, 226–233
- Derechin, M., Rustum, Y. M. & Barnard, E. A. (1972) *Biochemistry* **11**, 1793–1797
- Easterby, J. S. & Rosemeyer, M. A. (1972) *Eur. J. Biochem.* **28**, 241–252
- Feldman, I. & Kramp, D. C. (1978) *Biochemistry* **17**, 1541–1547
- Hoggett, J. G. & Kellett, G. L. (1976a) *Eur. J. Biochem.* **66**, 65–77
- Hoggett, J. G. & Kellett, G. L. (1976b) *Eur. J. Biochem.* **68**, 347–353
- Hoggett, J. G., Kellett, G. L. & Tickner, E. L. (1977) *Biochem. Soc. Trans.* **5**, 776–778
- Schmidt, J. J. & Colowick, S. P. (1973) *Arch. Biochem. Biophys.* **158**, 458–470 and 471–477
- Schulze, I. T. & Colowick, S. P. (1969) *J. Biol. Chem.* **244**, 2306–2316
- Tickner, E. L., Hoggett, J. G. & Kellett, G. L. (1976) *Biochem. Biophys. Res. Commun.* **72**, 808–815