Calorimetric Determination of Thermodynamic Parameters of Reaction Reveals Different Enthalpic Compensations of the Yeast Hexokinase Isozymes*

Received for publication, October 30, 2002, and in revised form, January 30, 2003 Published, JBC Papers in Press, February 28, 2003, DOI 10.1074/jbc.M211103200

M. Lucia Bianconi‡
From the Departamento de Bioquímica Médica, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-590, Brazil

The change in enthalpy and rate constants for the reactions of yeast hexokinase isozymes, PI (Hxk1) and PII (Hxk2), was determined at pH 7.6 and 25 °C by isothermal titration calorimetry. The reactions were done in five buffer systems with enthalpy of protonation varying from −1.22 kcal/mol (phosphate) to −11.51 kcal/mol (Tris), allowing the determination of the number of protons released during glucose phosphorylation. The reaction is exothermic for both isozymes with a small, but significant (p < 0.0001), difference in the enthalpy of reaction (ΔHR°), with an ΔHR° of −5.1 ± 0.2 (mean ± S.D.) kcal/mol for Hxk1, and an ΔHR° of −3.3 ± 0.3 (mean ± S.D.) kcal/mol for Hxk2. The Kₘ for ATP determined by ITC was very similar to those reported in the literature for both isozymes. The effect of NaCl and KCl, from 0 to 200 mM, showed that although the rate of reaction decreases with increasing ionic strength, no change in the ΔHR° was observed suggesting an entropic nature for the ionic strength. The differences in ΔHR° obtained here for both isozymes strongly suggest that, besides glucose phosphorylation, another side reaction such as ATP hydrolysis and/or enzyme phosphorylation is taking place.

In baker’s yeast, Saccharomyces cerevisiae, there are three enzymes that phosphorylate sugars: two hexokinases, PI (Hxk1) and PII (Hxk2), which catalyze the phosphorylation of glucose, fructose, and mannose; and a glucokinase specific for glucose and mannose. These enzymes are involved in the control of metabolism in yeast, but their individual roles are still unclear.

The wild-type strain of S. cerevisiae is able to adapt metabolism to growth conditions in response to the carbon source. Carbon catabolite repression is a transcriptional regulatory mechanism triggered by high concentrations of glucose and fructose, where the expression of a large number of genes necessary for the utilization of other carbon sources is repressed (1–4). It has been suggested that the triggering mechanism of catabolite repression is associated with the activity of Hxk1 and Hxk2 (2–5). Dynesen et al. (6) showed that glucose and fructose are equally capable of triggering catabolite repression. However, glucose repression requires Hxk2 (7), whereas either Hxk1 or Hxk2 is important for triggering fructose repression (2).

There is evidence that it is the presence of the protein and not the sugar kinase activity that acts as a signal for glucose repression (8–11). For instance, cell lines overexpressing glucokinase in a Hxk1/Hxk2 double-null mutant are insensitive to glucose repression whereas the phosphorylating activity increased 3-fold (10). Although mutant alleles with Hxk2 deletions at either the N terminus (Δ1–15) or the C terminus (Δ476–486) present low catalytic activity they were still fully functional in glucose signaling (11).

The Hxk2 is not only located in the cytosol but also in the nucleus in S. cerevisiae (1, 12–16). The nuclear localization of this enzyme seems to be involved in the formation of regulatory DNA-protein complexes necessary for signaling the glucose repression of the SUC2 gene, which encodes the secreted enzyme invertase that hydrolyzes sucrose and raffinose (15). The nuclear Hxk2 is also involved in the formation of specific DNA-protein complexes during glucose-dependent repression of these genes (12, 13, 15). Further evidence, using mutant cell lines expressing a truncated version of Hxk2 unable to enter the nucleus, showed that nuclear Hxk2 is necessary for glucose-induced repression signaling of HXK1 and GLK1 genes that encode for Hxk1 and glucokinase, respectively, and for glucose-induced expression of the HXK2 gene, which encodes the Hxk2 protein (15). Another protein, Med8p, was recently identified as a factor required for SUC2 gene expression in S. cerevisiae (18–20). This protein binds to downstream repressing sequences of the HXXK2 gene and to the upstream activating sequences of the SUC2 gene (18, 19). Recently, de la Cera et al. (20) showed that Hxk2 interacts specifically with Med8p both in vivo and in vitro, suggesting this interaction as a possible model of how Hxk2 is involved in glucose signaling.

Although the hexokinase isozymes play such different roles in yeast, 78% identity and around 90% homology in the amino acid sequence of Hxk1 (21) and Hxk2 (22) was found by using the Smith and Waterman algorithm (23). These enzymes are well characterized as homodimers of 52 kDa per subunit (24–26) with very similar tertiary structures (27–30). Each subunit of the yeast hexokinase isoforms consists of two domains separated by a deep cleft where glucose binds (27–30). Binding of glucose causes a 12° rotation of one domain related to the other, closing the cleft (30) and increasing the affinity for ATP (31, 32). At neutral pH, Hxk1 and Hxk2 are dimers, which can dissociate into monomers by increasing the pH or the ionic...
Thermodynamic of the Yeast Hexokinase Reaction

strength (33, 34). Despite of the great structural similarity of the yeast hexokinase isozymes, binding of glucose is strongly cooperative in the dimeric Hxk1 (35, 36) in contrast to the dimeric Hxk2 in which both sites are equivalent and binding is non-cooperative (36, 37). The equilibrium of association-dissociation of the yeast hexokinases seems to play an important role in the regulatory properties of these enzymes. It has been shown that under conditions of derepression both hexokinase isozymes are predominantly phosphorylated (38), lacking the ability for dimerization (39). Dephosphorylation can be induced by addition of glucose (38).

In this work, isothermal titration calorimetry (ITC) was used to determine the thermodynamic and kinetic parameters of the yeast hexokinase isozymes. ITC is a very sensitive technique based on the direct determination of the heat, absorbed or released, in a chemical reaction. The ITC results revealed that although the reaction is exothermic for both Hxk1 and Hxk2, there is a difference of 1.8 kcal/mol in their enthalpy of the reaction. Nevertheless, these isozymes have very similar behavior: they are both inhibited by phosphate and by increasing ionic strength, without a significant change in the reaction enthalpy. Studies with buffers of different enthalpy of ionization allowed the determination of the net number of protons released in the reaction.

EXPERIMENTAL PROCEDURES

Materials—The yeast hexokinase (ATP: 6-hexose 6-phosphotransferase, EC 2.7.1.1) isozymes, PI (type C-301) and PI1 (type C-302), were purchased from Sigma Chemical Co. as crystalline suspensions in ammonium sulfate. Prior to use, the ammonium sulfate was removed by centrifugation of the samples. After resuspension of the enzyme in the buffer used in the reaction medium, it was dialyzed for 2 h against the same buffer in the microdialyzer system 100 from Pierce to eliminate traces of ammonium sulfate. Glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) from Leuconostoc mesenteroides, NAD+, glucose, ATP, and the buffers (Tris, Mops, Hepes, and imidazole) were also from Sigma. MgCl₂ was from Merck Indústrias Químicas S.A., Brazil. All reagents were analytical grade.

Isothermal Titrination Calorimetry—The observed calorimetric enthalpies (ΔHobs) and the rate constants for the reaction of the yeast hexokinase isozymes were determined from thermograms (heat flow as a function of time) obtained in a MCS isothermal titration calorimeter from MicroCal Inc. (Northampton, MA) at 25 °C. The principles of implementation of ITC were described by Wiseman et al. (40). The reactions started by the injection of a small volume (7 μl) of the enzyme solution into the sample cell (volume, 1.38 ml) loaded with the reaction medium containing an excess of glucose and MgCl₂, but limited amounts of ATP. The final concentration of hexokinase in the calorimetric cell varied from 0.05 to 0.30 units/ml as indicated in the text. The Hxk1 and Hxk2 solutions were prepared in the same buffer of the calorimetric cell containing the reaction medium and can be described according to Equation 1,

\[ \frac{dQ}{dt} = -\Delta H \beta (d[P]/dt) \]

where \( \Delta H \) is the enthalpy change for the reaction, \( \beta \) is the volume of the calorimetric cell, and [P] is the concentration of product. Under the experimental conditions used here, the reaction can be described in terms of first-order kinetics in relation to ATP, and the heat flow is given by Equation 2,

\[ \frac{dQ}{dt} = -\Delta H \beta V (C_0 \exp(-\beta t) \exp(-kt)) \]

where \( \beta \) is the rate constant, and \( C_0 \) is the initial concentration of substrate (ATP).

The observed calorimetric enthalpy (ΔHobs) for the reaction of the yeast hexokinase isozymes was determined by dividing the total heat (Qₜ) released in the reaction by the amount of product formed. Qₜ was calculated by integrating the area under the peak of the calorimetric thermograms (dQ/dt as a function of time), and the amount of product formed (glucose 6-phosphate, Glc-6-P) in the assay was determined as described below. Data analysis was done with the Origin 5.0 software provided by MicroCal.

Assay for Hexokinase Activity—The kinetics of glucose phosphorylation by the yeast hexokinase isozymes was followed by measuring NADH formation at 340 nm in the coupled reaction with glucose-6-phosphate dehydrogenase. Data collection and analysis were done in a Hitachi U-2000 double-beam spectrophotometer equipped with a signal processing and control system that allows the calculation of rates of reaction using the time scan procedure. The assay media were the same used in the calorimetric experiments with the addition of G6PD (2.0 units/ml) and NAD⁺ (1.0 mM). The reactions were started by the addition of Hxk1 or Hxk2. The results were corrected for the dilution caused by the addition of G6PD and NAD⁺, absent in the calorimetric reactions. Enzymatic rates were determined by non-linear least square fits of the exponential growth of product formed with time, by monitoring the A₃₄₀ as a function of time.

Spectrophotometric Determination of Glucose 6-Phosphate—The amount of Glc-6-P formed in the reactions at the calorimeter was determined in parallel to the calorimetric measurements with an aliquot of the same volume of reaction medium used in the calorimetric cell (1.38 ml). The reaction was started after equilibrating the reaction medium in a water bath at 25.0 ± 0.3 °C at the same time as the reaction in the calorimeter cell was started by adding the same amount of hexokinase as that injected into the cell. After reaching the baseline and before the second injection in the calorimeter, the test tube was removed from the bath and placed in boiling water for 1 min in order to stop the reaction. It was then transferred to ice for at least 10 min before addition of G6PD and NAD⁺. After 20 min at 25.0 ± 0.2 °C, the amount of Glc-6-P was determined by measuring the A₃₄₀ considering that for each Glc-6-P, one NADH is formed. The molar extinction coefficient for NADH at 340 nm is 6,220 M⁻¹ cm⁻¹.

RESULTS

Calorimetric Determination of the Enthalpy of Reaction—The enthalpy of the yeast hexokinase reaction was determined by measuring the heat flow (μW/sec) as a function of time, following the injection of an enzyme solution into the calorimeter cell containing the reaction medium. The concentration of Hxk1 or Hxk2 varied from 0.05 to 0.2 units/ml, and all reactions were done with an excess of glucose and MgCl₂, but limited amounts of ATP, the only substrate totally consumed during the reactions. Fig. 1 shows a typical calorimetric trace obtained for the Hxk2 reaction at 25 °C in imidazole buffer. The downward displacement of the baseline after injection of the enzyme indicates the exothermic nature of the reaction. As the concentration of ATP decreases, the heat flow returns to the baseline level. After complete consumption of the ATP in the reaction medium, a second injection was done to calculate the heat of enzyme dilution (Fig. 1).

The area under the peak corresponds to the total heat (Qₜ) released in the reaction (42). The heat of dilution (Qd) could be minimized to 0.4–0.5% of Qₜ by preparing the enzyme solution in the same buffer as that used in the reaction medium. The observed calorimetric enthalpy (ΔHcal) was calculated by divid-
The reaction was done in 50 mM imidazole buffer at 24.97 °C with the enzyme at a final concentration of 0.2 units/ml. The reaction started after equilibration at the desired temperature by the injection of 14 μl of the Hxk2 solution into the calorimetric cell containing the reaction medium (10 mM glucose, 5 mM MgCl₂, and 0.1 mM ATP in 50 mM Mops buffer). Following the injection of the enzyme, an exothermic reaction started as negative values of the heat flux is observed. A total consumption of the ATP occurs, as observed by the return of the heat flux to the baseline level. The time for the first and second injections of Hxk2 solution is indicated by arrows. The area under each peak gives the total heat (Qₜ, in μcal) for the reaction (1st injection) and for the dilution of the enzyme (2nd injection).

The calorimetric enthalpy is the sum of different heat effects taking place during the reaction. If the reaction involves the release (or uptake) of protons, for instance, ΔHcal will be a combination of the intrinsic enthalpy of reaction (ΔHᵢ), and the enthalpy of protonation (or ionization) for each proton absorbed (or released) by the buffer used. In the reaction catalyzed by hexokinases the phosphorylation of glucose is accompanied by a release of H⁺ to the solution, which, in turn, is absorbed by the buffer. The relationship between ΔHᵢ and the enthalpy of buffer protonation (ΔHᵢ) can be expressed by Equation 3,

$$\Delta H_{\text{cal}} = \Delta H^i + n \Delta H^p$$  (Eq. 3)

where n represents the number of protons released in the reaction and absorbed by the buffer, and ΔHᵢ is the intrinsic enthalpy of reaction.

Different buffer systems with ΔHᵢ ranging from −1.22 kcal/mol (phosphate) to −11.38 kcal/mol (Tris) at 25 °C (43) were chosen to study the reaction of the yeast hexokinases, and the correlation between ΔHᵢ and ΔHᵢ for both isozymes is shown in Fig. 2. From these curves, it was found that although the reaction is exothermic for either Hxk1 or Hxk2, a small difference in ΔHᵢ was found. The intercept gives a ΔHᵢ of −5.13 ± 0.24 kcal/mol for Hxk1 and ΔHᵢ of −3.34 ± 0.27 kcal/mol for Hxk2. Nevertheless, the number of protons released during the glucose phosphorylation reaction is essentially the same for Hxk1 (n = 0.94) and Hxk2 (n = 0.96).

In those experiments, it was found that both isozymes were inhibited in phosphate buffer. On the contrary, the rate of reaction was unaffected with Hepes, imidazole, Mops, or Tris at a final concentration of 50 mM. In order to determine the ΔHᵢ in phosphate buffer, the concentration of enzyme was increased to 0.3 units/ml, and the thermograms were recorded for a longer period of time to assure the total consumption of the ATP in the medium. A decrease in the buffer concentration from 50 to 10 mM was also important to attenuate the inhibitory effects of phosphate.

Calorimetric Determination of Kinetic Parameters—The integrated heat (Q) as a function of time (Fig. 3) shows that the rate of the reaction measured by calorimetry is perfectly comparable to the spectrophotometric assay done in a reaction medium with the same final concentration of reagents of that used in the calorimetric cell. The initial rate of reaction was determined as described under “Experimental Procedures.” As expected from the similar kinetics observed by the two methods employed, the rate of reaction determined by calorimetry was proportional to the amount of enzymes added (Fig. 4A). The initial velocities found from the calorimetric experiments at constant enzyme concentration (0.05 units/ml) and ATP varying from 0.05 to 0.2 mM with saturating concentrations of glucose and Mg²⁺, allowed the determination of the $K_m$ for ATP (Fig. 4B). The $K_m$ values obtained from the calorimetric assays for Hxk1 ($K_m$, 155 μM) and Hxk2 ($K_m$, 210 μM) are very similar to those reported in the literature (31, 32).

Effect of Ionic Strength in the Reaction Rate and Enthalpy—The effect of ionic strength in the rate of reaction of the yeast hexokinases was studied in 10 mM buffer (Hepes, Mops, imidazole, or Tris) with NaCl or KCl ranging from 0 to 200 mM. Phosphate buffer was not used in this study because of the inhibitory effects on the hexokinase isozymes discussed before.
strength with a maximum at 50 mM KCl, while the rate of reaction has a bell-shaped dependence with ionic strength (Fig. 5A) for the reaction catalyzed by Hxk1 as determined by calorimetric assays at 25.04 ± 0.05 °C. The assays were done in 50 mM Mops buffer, pH 7.6, containing 10 mM glucose, 5 mM MgCl₂, where the ATP concentration was 0.1 mM in A and varied from 0.05 to 1.0 mM in B.

Very similar results were found, either with KCl or NaCl, with the other buffers used here. The rate of reaction decreases with the increase in ionic strength as observed in the calorimetric (Fig. 5A) and in the spectrophotometric (Fig. 5B) assays, where the maximum activity was observed in the absence of salt (10 mM buffer). However, ΔH°cal, and consequently ΔH°fold, did not change over the salt concentration used here, showing that this parameter is independent of ionic strength (Fig. 5C).

This result is very similar to those obtained by Morin and Freire (42) for the reaction with cytochrome c oxidase, where the rate of reaction has a bell-shaped dependence with ionic strength with a maximum at 50 mM KCl, while the ΔH°fold does not change with the addition of up to 200 mM KCl. For each case, yeast cytochrome c oxidase and hexokinase isozymes, the decrease on the reaction rate together with the lack of dependence of ΔH° with the ionic strength suggests an entropic nature for these effects.

Takahashi et al. (44) showed by differential scanning calorimetry (DSC) that the conformation of the yeast Hxk2 is affected by the ionic strength. In the absence of glucose and at low ionic strength, the thermal unfolding of Hxk2 is characterized by a double-peaked endotherm, resulting from the independent unfolding of the two structural domains of the protein. However, with 200 mM NaCl there is a destabilization of the more stable of the two domains so that both domains unfold as a single unit (44). Interestingly, the strong effect of NaCl on the interaction between the two domains of the protein is not accompanied by any significant change in the calorimetric enthalpy of unfolding.

In this work, it was observed that the heat of enzyme dilution (Qd), obtained from the second injection of enzyme, increased in the reaction media containing KCl or NaCl. In order to study the effects of salt in the conformation of the hexokinases by using ITC, several injections of the enzymes in solutions containing different concentrations of KCl or NaCl were done. The enzyme was prepared in 10 mM buffer and injected in solutions prepared in the same buffer but containing 0, 50, 100, 150, or 200 mM salt. Control experiments were performed by injecting 10 mM buffer in solutions containing variable concentrations of salt. The heat of dilution of the enzyme in 10 mM buffer was exothermic (Qd is $-1.36 ± 0.12$ kcal/mol for Hxk1, and $-1.19 ± 0.07$ kcal/mol for Hxk2). In contrast, the transfer of the enzyme at low ionic strength to the cell containing a high ionic strength solution was always endothermic, without significant difference in the range of NaCl or KCl concentrations used here. Thus, Qd was $+1.48 ± 0.25$ kcal/mol for PI and $+1.57 ± 0.4$ kcal/mol for PII. Again, as observed with the heat of reaction, there is no enthalpic change induced by increasing ionic strength. This result is consistent with DSC studies (44), which showed that although NaCl causes a great effect on the interaction between domains in the yeast hexokinase, this is not accompanied by any significant change in the calorimetric enthalpy of the thermal transition. Enthalpic effects are usually associated with changes in the secondary structure of a protein. Thus, our results indicate that the increase in ionic strength does not induce a major rearrangement on the secondary structure of these isozymes. It is possible that the rate of reaction decreases because of the effect of increasing ionic strength on the equilibrium of association-dissociation of the subunits (34). However, the influence of salt on the catalytic rate constant can be explained by the destabilization of one of the domains as observed by Takahashi et al. (44).
DISCUSSION

The determination of kinetic parameters is essential to characterize enzymes of biological interest. However, the direct measurement of either reagents or products is not always possible by optical or electrochemical assays. Substrate modification by introducing an optically active group can change the kinetic properties of the enzyme, while coupled reactions can also introduce undesirable errors. Isothermal titration calorimetry is a very sensitive technique for the determination of both thermodynamic and kinetic parameters of enzymatic reactions, based on the measurement of the heat, absorbed or released, in a chemical reaction of binding, dilution, or transformation. ITC has been used for the determination of thermodynamic (41, 42, 45–47) as well as kinetic (41, 48–52) parameters of different enzymes.

The kinetic parameters found here are in agreement with those reported in the literature showing that ITC is suitable for this kind of study. The advantage of using ITC to study the catalytic behavior of hexokinases is that reaction rates can be determined directly without using the coupled reaction with G6PD. This is especially important when the purpose of a particular study involves the effect of activators, inhibitors, or stabilizers. It is important that these compounds will affect only the enzyme of interest and not the other used in the coupled reaction.

The ionic strength dependence of the yeast hexokinase activity can be related to different causes. It has been shown that increasing ionic strength affects the monomer-dimer equilibrium, favoring the dissociation to monomers (34, 35). Therefore, the results found here are indicative that the decrease in the activity of both yeast Hxk1 and Hxk2 is probably a result of the dissociation of the dimers. However, the decrease in activity can be due to the fact that NaCl causes a destabilization of the more stable domain as seen by DSC studies (44). Nevertheless, our data indicate that the ionic strength effects are of entropic nature.

However, the most intriguing result obtained here is related to the enthalpy of the reaction. A difference of 1.8 kcal/mol was found in the enthalpy for the reaction catalyzed by the two hexokinase isozymes from yeast, after taking into account the effects of buffer protonation. Statistical analysis by the Student’s t test shows that the difference in heat for the reaction with each isozyme has a high significance level with p < 0.0001. The reaction is exothermic for both Hxk1 and Hxk2. If the heat effects determined as ΔH° obtained here may contain other contributions that are not being considered. It is important to remember that ΔH° is, in fact, a sum of the changes in enthalpy from every single event, reflecting the changes in heat for the whole system. Our results strongly suggest that, besides glucose phosphorylation, another side reaction is taking place. Therefore, the difference in the calculated ΔH° could be either by (i) enzyme phosphorylation (38) or (ii) ATP hydrolysis (17, 32). The first usually occurs under conditions of derepression, in vivo. The latter is accounted by an ATPase activity of the yeast hexokinase. Although the ATP hydrolysis reaction is several times slower than glucose phosphorylation, it can be increased by lowering the water activity in the medium (17). Therefore, the heat due to reaction (i) or (ii), or a combination of both, will sum up to the heat for the transfer of a phosphate group to glucose resulting in the observed differences in ΔH°.

In short, this work suggests that in the overall catalyzed reaction each hexokinase isozyme has a different enthalpic compensation during glucose phosphorylation, which can be important under in vivo conditions. This can explain, in part, the need for two very similar isozymes with different behavior in the control of metabolism in yeast.

Acknowledgments—We thank MicroCal, LLC. for technical support and advice, Dr. L. de Meis for calorimetric facilities (maintained by funds from PRONEX-CNPq), and A.C. Miranda for technical assistance.

REFERENCES
1. de Meis, L., Bianconi, M. L., and Suzano, V. A. (1997) Biochim. Biophys. Acta 133, 127–134
2. Bogus, A. B., and Franchek, D. G. (1990) Eur. J. Biochem. 190, 371–375
3. Krieger, T. M., Rush, J., Bogus, A. B., Clifton, D., and Fraenkel, D. G. (1994) Biochemistry 33, 3484–3485
4. Millar, N. C., Howarth, V., and Gutfreund, H. (1987) Biochem. J. 240, 485–488
5. Williams, B. A., and Toone, E. J. (1993) FEBS Lett. 350, 147–150
6. Kriegel, T. M., Rush, J., Vojtek, A. B., Clifton, D., and Fraenkel, D. G. (1994) Biochemistry 33, 3484–3485
7. Millar, N. C., Howarth, V., and Gutfreund, H. (1987) Biochem. J. 240, 485–488
8. Krieger, T. M., Rush, J., Bogus, A. B., Clifton, D., and Fraenkel, D. G. (1994) Biochemistry 33, 3484–3485
9. Williams, B. A., and Toone, E. J. (1993) FEBS Lett. 350, 147–150
10. Kriegel, T. M., Rush, J., Vojtek, A. B., Clifton, D., and Fraenkel, D. G. (1994) Biochemistry 33, 3484–3485
11. Millar, N. C., Howarth, V., and Gutfreund, H. (1987) Biochem. J. 240, 485–488
12. Krieger, T. M., Rush, J., Bogus, A. B., Clifton, D., and Fraenkel, D. G. (1994) Biochemistry 33, 3484–3485
13. Millar, N. C., Howarth, V., and Gutfreund, H. (1987) Biochem. J. 240, 485–488
14. Krieger, T. M., Rush, J., Bogus, A. B., Clifton, D., and Fraenkel, D. G. (1994) Biochemistry 33, 3484–3485
15. Millar, N. C., Howarth, V., and Gutfreund, H. (1987) Biochem. J. 240, 485–488
Calorimetric Determination of Thermodynamic Parameters of Reaction Reveals Different Enthalpic Compensations of the Yeast Hexokinase Isozymes

M. Lucia Bianconi

J. Biol. Chem. 2003, 278:18709-18713.
doi: 10.1074/jbc.M211103200 originally published online February 28, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M211103200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 49 references, 7 of which can be accessed free at http://www.jbc.org/content/278/21/18709.full.html#ref-list-1