Urea and Methylamine Effects on Rabbit Muscle Phosphofructokinase

CATALYTIC STABILITY AND AGGREGATION STATE AS A FUNCTION OF pH AND TEMPERATURE*

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The effects of urea and several methylamine solutes on the catalytic stability and aggregation properties of rabbit muscle phosphofructokinase were assessed at physiologically realistic concentrations of the solutes under several pH and temperature regimes. The loss of catalytic activity observed under conditions of pH-induced cold lability was significantly reduced in the presence of trimethylamine-N-oxide, N-trimethylglycine and N-methylglycine (order of decreasing effectiveness). The concentration-dependent methylamine stabilization of the enzyme, seen with as little as 50 mM trimethylamine-N-oxide, was accompanied by increased aggregation of the enzyme to molecular weights greater than the tetramer (polytetramer) as solute concentration was raised to 400 mM.

At pH 6.5-8.7 and 25 °C, concentrations of urea greater than 25 mM promoted a time-dependent inactivation of the enzyme which was enhanced at lower temperatures. The urea sensitivity of the enzyme exhibited a strong pH dependence; preincubation of the enzyme with 0.8 M urea for 1 h at pH 8.0 did not result in measurable inactivation. The fluorescence emission wavelength maximum of the enzyme was shifted to longer wavelengths and the fluorescence intensity was increased as pH was lowered to 7.0, suggesting the occurrence of a protein conformation change. Specific amino acid residues of the tetramer became protonated. Measurements of enzyme light scattering indicated that perturbation by urea was correlated with tetramer dissociation, which was reversible by dialysis at 25 °C. The urea and methylamine influences on phosphofructokinase activity and structure were not counteracting.

The synergistic interactions among pH, temperature, and solutes observed with phosphofructokinase are compared to effects on other associating-dissociating protein systems in order to evaluate possible mechanisms of action of these low molecular weight solutes.

The structural and functional properties of enzymes frequently are strongly influenced by low molecular weight solutes which are not substrates, cofactors, or allosteric modulators of the enzymes. For example, neutral inorganic ions of the Hofmeister (lyotropic) series (1, 2) have pronounced effects on enzyme conformation and subunit assembly (3, 4) and catalytic activity (4-7), as do certain naturally occurring organic solutes such as glycerol (8, 9), sucrose (10), and urea (11). Extending our knowledge of these protein-solute interactions is important for several reasons. First, the basic mechanisms of protein-solute interactions remain poorly understood and it is not clear, for example, whether inorganic salts and organic solutes influence proteins by similar or different mechanisms. Second, the physiological roles which may be played by solute influences on protein structure and function have received relatively little attention. In view of the wide diversity of organisms known to contain intracellular organic solute concentrations in the range of approximately 0.5 to 3 M, including many marine animals (12, 13), halotolerant plants (14, 15), and bacteria (16), it is appropriate to inquire about the regulatory roles played by these solutes and the possible synergisms which may take place between these solutes' effects and changes in other parameters such as pH and body temperature.

We have approached these issues through examining solute, pH, and temperature interactions with rabbit muscle phosphofructokinase (EC 2.7.1.11), an enzyme noted for its functional and structural sensitivities to these variables (17-21). We have placed special emphasis on the effects of physiologically realistic concentrations of urea and a family of methylamines: trimethylamine-N-oxide, N-trimethylglycine (betaine), and N-methylglycine (sarcosine). These nitrogenous solutes occur at concentrations of several tenths molar in many marine animals (13, 22) and some plants (15), and recently studies have demonstrated that the methylamine solutes are potent counteractants of urea perturbation of enzyme structure and function (11, 22). To further examine these effects, we have studied the kinetic stability and aggregation properties of phosphofructokinase, focusing on the following set of questions. First, how effective are physiologically realistic concentrations of urea and the methylamine solutes on this enzyme? Do these nitrogenous solutes influence phosphofructokinase via mechanisms similar to or different from those associated with neutral salt effects, and are urea and methylamine influences counteracting? Are there synergistic effects between the influences of the nitrogenous solutes and the influences of pH and temperature? Last, are there apparent roles for these solute, pH, and temperature effects on phosphofructokinase in organisms, for example, hibernating and estivating animals and cartilaginous fishes (sharks, skates, and rays), where high concentrations of urea and/or methylamines are present in the intracellular fluids?

EXPERIMENTAL PROCEDURES

Chemicals—Phosphofructokinase was either obtained as a crystalline suspension in ammonium sulfate (type III) from Sigma Chemical Co. or purified from rabbit skeletal muscle as described below. Glycerol-3-phosphate dehydrogenase (type III) from Sigma Chemical Co. was purified from rabbit skeletal muscle as described below. Glycerol-3-phosphate dehydrogenase (rabbit muscle) was a product of Boehringer Mannheim. Aldolase (type I), triose phosphate isomerase (type III), Sepharose 4B, CNBr-activated Sepharose 4B, N-[6-(aminomethyl)-carbamoyl-methyl]ATP, and N-acetyltryptophanamide were all purchased from Sigma Chemical Co. Solutions of trimethyl-

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amine-N-oxide dihydrate (Eastman Kodak Co.) were either passed through a Millipore filter (0.45-µm pore size) or centrifuged at 60,000 × g (15 min) to remove undissolved particulates before use. Urea (Sigma) was treated with Amberlite MB-3 mixed bed ion exchange resin to remove isocyanate and ammonium ions. All other biochemicals were reagent grade and used as received from the supplier.

**Stabilization of Phosphofructokinase**—Phosphofructokinase was purified from both fresh and frozen rabbit skeletal muscle according to the method of Ramadoss et al. (23), with the following modifications. When frozen tissue was used (flash frozen in liquid nitrogen and stored at −20 °C for a maximum of 2 months), the phosphofructokinase was first activated and solubilized (24). Muscle homogenates, prepared in an ice-cold solution of 30 mM KF and 1 mM EDTA, were centrifuged at 25,000 × g for 30 min, and the supernatant was utilized for the subsequent ultracentrifugation step. The pellet fractions were dissolved in 50 mM Tris phosphate buffer (pH 8.0) containing 0.2 mM EDTA and 5 mM dithiothreitol by stirring for 1 h at room temperature. Any undissolved material was removed by centrifugation. The preparation was applied to a N-[(6-aminohexyl)-carbamoylmethyl]-ATP-Sepharose-4B matrix with a 5-ml loading technique. After the washing procedure (23), elution of phosphofructokinase was accomplished with a mixture of 0.14 mM fructose 6-phosphate and 0.1 mM ADP. At this stage, the enzyme was not homogeneous, but required an additional heat treatment step. The enzyme (3.5 mg/ml) was incubated at 55 °C for 5 min in 0.1 M Na-phosphate buffer (pH 8.0) containing 1 mM EDTA and 5 mM dithiothreitol; the procedure resulted in a negligible loss of activity. This purification protocol yielded approximately 8–10 mg of purified phosphofructokinase from 100 g (wet weight) of rabbit skeletal muscle.

Polyacrylamide gel electrophoresis was performed following the method of Laemmli (25). The 12% acrylamide gels were heavily loaded with phosphofructokinase (100 µg/ml) and electrophoresed in the presence of sodium dodecyl sulfate. Densitometry tracings of the gels stained with fast green showed the phosphofructokinase preparation to be at least 95% pure. The enzyme was stored as a precipitate (5–10 mg/ml) in 70% (NH₄)₂SO₄, prepared in 0.1 M phosphate buffer (pH 8.0) containing 1 mM EDTA and 5 mM dithiothreitol; the procedure resulted in a negligible loss of activity. For all experiments, a 2-nm band pass. After Millipore filtration, readings of experimental solutions were made in the same cuvette with and without phosphofructokinase so that the net scatter of the added protein could be determined. Phosphofructokinase at 0.2 mg/ml gave an acceptable level of amplification for use when the dependence inactivation studies, enzyme aliquots were removed from the cuvette and assayed for catalytic activity.

**Intrinsic Fluorescence and Light-scattering Measurements**—The fluorescence emission spectrum of purified phosphofructokinase (0.2 mg/ml) was determined in 0.1 M phosphate buffer (pH 8.0) containing 5 mM dithiothreitol at the indicated pH, temperature, and solute concentrations. Measurements were performed using a Perkin-Elmer model MPF-44A fluorescence spectrophotometer equipped with a thermostatted cuvette holder. An excitation wavelength of 285 nm was used, and both excitation and emission bandwidths were set at 10 nm. Spectra were not corrected for the spectral responses of the instrument. All solutions were passed through a Millipore filter (0.45-µm pore size) to remove any dust particles prior to measurement.

Light-scattering measurements were made utilizing the 90° geometry of the spectrofluorometer above, but in this case, excitation and emission monochromators were set at 360 and 420 nm, respectively. The ratio of the two intensities was measured against a standard set to 1.0. Some kinetic measurements were made using phosphofructokinase treated with activated charcoal (Norit A) as described by Liou and Anderson (27); the results were identical with those with the untreated enzyme. The specific activity of the purified phosphofructokinase was approximately 130 units/mg of protein at 25 °C. One unit is defined as the production of 1 µmol of fructose 1,6-bisphosphate/minute (one-half the measured rate of the enzymatically coupled NAD⁺ production). Protein was assayed according to the method of Lowry et al. (28), as modified by Peterson (29).

**Inactivation Experiments in the Presence of Nitrogenous Solutes**—Studies centering on the ability of membrane solutes to stabilize phosphofructokinase catalytic activity under conditions of pH-induced cold lability employed the frontal elution technique described by Winzor (33). With this procedure, the enzyme solution (0.2 mg/ml) was applied continuously to the column until the protein concentration (or catalytic activity) in the effluent was the same as that of the applied sample. The midpoint of the elution volume was defined as the protein concentration equivalent to the elution volume obtained by conventional gel filtration procedures. Experiments were performed at 3 °C using a Sepharose 6B (0.75 × 28 cm) (40-120-µM bead size) column equilibrated with either 0.1 M phosphate buffer (pH 8.0, 0.2 mM dithiothreitol) or the same buffer containing 400 mM trimethylamine-N-oxide. The bed volume of the column was constant during all experiments and showed no shrinkage or swelling due to the choice of equilibration buffer. Constant volume fractions (0.15 ml) were collected by drop count. A Gilson FC-100 microfractionator; flow rate was 9 ml/h.

**RESULTS**

**Solute Stabilization of Phosphofructokinase Catalytic Activity**—Under conditions of pH-induced cold lability (pH 6.5, 6 °C), both the rate of loss and the absolute loss of phosphofructokinase catalytic activity are substantially reduced in the presence of membrane solutes. The stabilizing effect is...
shown in Fig. 1, which compares the time course of enzyme inactivation in the presence and absence of trimethylamine-N-oxide, betaine, and sarcosine (order of decreasing protective ability). The degree of enzyme stabilization is correlated with the extent of methylation of the solute’s nitrogen atom. First order plots of the data of Fig. 1 show that loss of activity is not strictly a first order process, supporting the earlier finding of Bock and Frieden (18, 26), who described the inactivation as at least biphasic.

The protective methylamine effect is observed at unusually low concentrations of these solutes when compared to solute concentrations typically used to achieve protein stabilization. A plot of residual phosphofructokinase activity after a 60-min preincubation in the presence of different trimethylamine-N-oxide concentrations (Fig. 2) shows a concentration threshold of approximately 50-100 mM for the stabilization effect. Maximal stabilization is reached at approximately 1 x trimethylamine-N-oxide. A parallel experiment conducted with two neutral salts, KCl and KF, allowed a comparison of particular interest to be made. These strong electrolytes have effects on enzymic activity and structural stability which are related to the salt’s positions in the Hofmeister series (1). KF, a salting-out salt (decreases protein group solubility), stabilizes phosphofructokinase against cold inactivation (Fig. 2), while KCl, which has little effect on protein group solubility, destabilizes the enzyme. Although both trimethylamine-N-oxide and KF stabilize phosphofructokinase activity, the effective concentrations for the two solutes are markedly different. The much lower concentration at which trimethylamine-N-oxide exhibits stabilizing influences suggests a different mechanism of action from that of KF.

Inactivation of Phosphofructokinase by Low Concentrations of Urea—Preincubation of phosphofructokinase at 25 °C and pH 6.5 with as little as 25 mM urea causes a significant inactivation of the enzyme (Fig. 3, bottom). Fig. 3 (top) shows that the inactivation in the presence of 50 mM and 100 mM urea is time-dependent and, based on semilogarithmic plots (not shown), the inactivation kinetics closely resemble those for cold lability (Fig. 1). The sensitivity of the enzyme to urea...
can be altered by addition of substrates (Fig. 3, bottom) and by changing the enzyme concentration (Fig. 4) present during the incubation. Fructose 6-phosphate (0.1 mm) protects the enzyme slightly against urea inactivation, while ATP (2 mm) accentuates the activity loss (Fig. 3, bottom). Consistent with a previous report (19), control studies showed that fructose 6-phosphate and ATP affect phosphofructokinase stability at pH 6.5 in the absence of urea, but the combined effects of the substrates and urea were not additive. The extent of inactivation is reduced as the enzyme concentration is increased from 0.02 mg/ml to 1.0 mg/ml (Fig. 4), suggesting the possibility of enzyme dissociation during the inactivation process.

Another characteristic of the urea perturbation which is again similar to the low temperature inactivation (Fig. 1) is a strong pH dependence (Table I). Preincubation of the enzyme at pH 8.0 in the presence of up to 0.8 M urea had no effect on catalytic activity. However, when pH was lowered slightly below 7.0, a condition common in skeletal muscle (34), inactivation by urea was observed and was more extensive at reduced temperatures.

The inactivation induced by urea was not offset by the presence of trimethylamine-N-oxide at a concentration ratio of 2:1 ([urea]:[trimethylamine-N-oxide]) to test for its ability to counteract urea effects. This ratio was found to be optimally effective in offsetting urea perturbations of a variety of other enzyme kinetic (22) and structural (11) phenomena.

The urea-induced loss of catalytic activity did not lead to irreversible denaturation of the enzyme; the inactivation observed after incubation in the presence of 400 mM urea was reversed by dialysis at room temperature (Table II).

Fluorescence and Light-scattering Studies—To investigate the structural bases of the losses in catalytic activity, we performed a series of fluorescence and light-scattering studies. Table III lists the wavelength maxima of the fluorescence emission spectrum ($\lambda_{\text{max}}$) as a function of pH at 6°C. As pH was lowered from 8.0 to 6.7, a 2-nm red shift occurred in the $\lambda_{\text{max}}$ of the purified enzyme. Addition of 400 mM trimethylamine-N-oxide produced a further red shift in $\lambda_{\text{max}}$ (data not shown). Accompanying these red shifts were consistent increases in the maximum fluorescence intensity. Addition of urea (up to 400 mM) did not significantly alter the $\lambda_{\text{max}}$ over the same pH range.

While these changes in fluorescence properties of phosphofructokinase could derive from pH- and solute-induced alterations in enzyme conformation, with concomitant changes in the polarity of the environments of tryptophyl residues, it is also conceivable that direct interactions between solutes and tryptophyl could influence the enzyme’s fluorescence characteristics without any change in conformation occurring. We investigated this latter possibility by studying the effects of pH and trimethylamine-N-oxide on the fluorescence properties of a model tryptophyl compound, N-acetyltryptophan-
amide (NATA). Varying buffer pH between 6.5 and 8.0 had no effect on the \( \lambda_{\text{max}} \) and fluorescence intensity of NATA. Therefore, the pH-induced shifts in \( \lambda_{\text{max}} \) and fluorescence intensity of the enzyme presumably are due to a conformational change in the enzyme. Trimethylamine-N-oxide causes a concentration-dependent blue shift in the \( \lambda_{\text{max}} \) of NATA (3 nm with 800 mM trimethylamine-N-oxide), accompanied by an increase in fluorescence intensity. The small red shift in the \( \lambda_{\text{max}} \) of phosphofructokinase in the presence of 400 mM trimethylamine-N-oxide cannot be explained by the solute's effects on the tryptophyl fluorescence, but instead may find an explanation in terms of solute-induced change in tryptophyl environment(s).

Laid et al. (35) and others (17, 26) have shown a good correlation between light scattering by phosphofructokinase and both aggregation state and specific activity. Consequently, light-scattering studies were performed in the presence of nitrogenous solutes to look for possible time-dependent changes in enzyme structure which were not detected in the fluorescence studies. A decrease in phosphofructokinase light scattering is indicative of the formation of lower molecular weight species of the enzyme (17, 35). At concentrations greater than 0.2 mg/ml, at pH 8.0, rabbit muscle phosphofructokinase exists as a mixture of high molecular weight polymers (polytetramers) which are in rapid equilibrium with the tetramer (35). At concentrations of 0.2 mg/ml and lower, the dominant enzyme species is the tetramer (17, 31, 32, 36), but the tetramer can be induced to dissociate into inactive dimers under certain conditions (26, 35).

Under conditions favoring enzyme stability (pH 8.0 at 6 °C), the light scattering and catalytic activity of phosphofructokinase (0.2 mg/ml) are stable with time (Fig. 5). When the pH is lowered to 6.7, creating conditions for cold inactivation, a decrease in light scattering occurred, a result consistent with the findings of Bock and Frieden (26) and suggestive of dimer formation. In the presence of 100 mM trimethylamine-N-oxide, the decrease in light scattering is reduced, as is the extent of inactivation. With 400 mM trimethylamine-N-oxide phosphofructokinase, light scattering is initially increased 20–25% above the control (pH 8.0, no trimethylamine-N-oxide) values and then slowly decreases with time. The increase in light scattering with 400 mM trimethylamine-N-oxide is the same magnitude above the control at both pH 6.7 and 8.0. However, as would be predicted, the light scattering of the enzyme at pH 8.0 in 400 mM trimethylamine-N-oxide does not exhibit a time-dependent decrease (data not shown). Since control values for phosphofructokinase (0.2 mg/ml) light scattering should reflect those of a molecular population consisting predominantly of tetramers, these data suggest that 400 mM trimethylamine-N-oxide favors aggregation of the enzyme to high molecular weight (polytetramer) species.

Results of analogous experiments in the presence of urea are presented in Fig. 6. The inactivation observed with urea

\[ \text{FIG. 5. Simultaneous measurements of phosphofructokinase light scattering (top) and catalytic activity (bottom) with or without trimethylamine-N-oxide as a function of time. Preincubations of the enzyme (0.2 mg/ml) were conducted under conditions of either pH-induced cold lability (pH 6.7, 6 °C) or conditions promoting a stable tetramer (pH 8.0, 6 °C). ○, preincubations at pH 8.0 without trimethylamine-N-oxide; ●, pH 6.7, without trimethylamine-N-oxide; □, pH 6.7, with 100 mM trimethylamine-N-oxide; △, pH 6.7, with 400 mM trimethylamine-N-oxide.} \]

\[ \text{FIG. 6. Simultaneous measurements of phosphofructokinase light scattering (top) and catalytic activity (bottom) with or without urea as a function of time. Preincubations of the enzyme (0.2 mg/ml) were conducted at 25 °C and at the indicated pH values.} \]

\[ \text{FIG. 7. Frontal elution gel filtration of phosphofructokinase (0.2 mg/ml) on Sepharose 6B in the presence (○) or absence (●) of 400 mM trimethylamine-N-oxide.} \]
stabilize the intramolecular conformations of proteins. Blages are stabilized by the same types of weak bonds that accompany association-dissociation phenomena. Protein assembly reactions like the polymerization of actin, for example, and suggest that the small number of weak interactions responsible for stabilizing polymers may be unusually sensitive to low concentrations of solutes.

In the presence of urea and methylamines with specific sites on this enzyme. A similar conclusion was offered for the deoxygenated sickle cell hemoglobin polymer which is particularly sensitive to guanidinium series phenomena is that the urea and methylamine cold lability was found at concentrations as low as 100 mM, while stabilization by KP (a salting-out Hofmeister salt) was not found until nearly 1 M (Fig. 2). An implication of this sensitivity to urea and methylamines is that the tetrameric and polytetrameric states of the enzyme are maintained via very low stabilization free energies. This conclusion is of course consistent with models of phosphofructokinase structure-function relationships which emphasize the potential for rapid interconversion between polytetrameric, tetrameric, and dimeric states in response, for example, to alterations in pH and concentrations of allosteric regulators (17-20, 46, see below). Other protein assembly reactions like the polymerization of sickle cell hemoglobin (47) and association-dissociation of sesamé α-globulin (3) also display extremely small free energy changes. Addition of inorganic salts (0.2 M) to sickle cell hemoglobin solutions only altered the free energy change associated with depolymerization (3.54 kcal/mol without added salt) by 0.4 kcal/mol, but it had pronounced effects on the equilibrium of the reaction. These low stabilization ∆G values are in marked contrast to those accompanying the isothermal unfolding of ribonuclease (16-20 kcal/mol) (48), for example, and suggest that the small number of weak interactions responsible for stabilizing polymers may be unusually sensitive to low concentrations of solutes.

The second observation which is inconsistent with Hofmeister series phenomena is that the urea and methylamine effects on phosphofructokinase were not counteracting. This finding contrasts with several other cases in which a 2:1 molar ratio (the ratio typically observed in cells which accumulate these two solute(s) (22) led to essentially no effects on protein structure (11) and function (22). These counteracting effects, noted with solute concentrations of several tenths molar, may be effected by solubility mechanisms as discussed earlier. However, we propose that the low concentration effects found with phosphofructokinase may derive from the interactions of urea and the methylamines with specific sites on this enzyme. A similar conclusion was offered for the deoxygenated sickle cell hemoglobin polymer which is particularly sensitive to guanidinium salts (<0.2 M). It was suggested that since the guanidinium moiety corresponds to the side chain functional group of arginine, guanidium could competitively inhibit the formation of one or more salt bridges involving arginine at intermolecular contact sites in the polymer (47). The perturbation by guanidinium could not be counteracted by the addition of stabilizing solutes.

Factors Modulating the Solute Sensitivity of Phosphofructokinase—In addition to increased regulatory behavior and...
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kinetic complexity exhibited by phosphofructokinase as pH is lowered, urea perturbation of the enzyme and susceptibility to cold inactivation become greatly accentuated below pH 7. The appearance of regulatory kinetics is likely due to protonation of specific protein residues (presumably histidyls (18–20)) and subsequent preferential binding of ligands to the protonated or unprotonated forms of the enzyme (31, 49, 50). In the absence of ligands, the equilibrium constant for the ratio of protonated to unprotonated forms is defined by temperature and pH (50). The protonated tetramer is susceptible to dissociation into dimers as temperature is lowered, and it has been concluded that the ability of regulatory ligands to alter the degree of phosphofructokinase cold lability is dependent on pK changes of histidyl residues which, in turn, are due to conformational changes occurring upon ligand binding (19). A similar explanation is plausible for the ligand influences seen in this study on urea sensitivity of phosphofructokinase (Fig. 3).

The precise reason for the accentuated tendency of the protonated tetramer to dissociate to dimers is not clear, but the small red shift in $\lambda_{\text{max}}$ and the increase in fluorescence intensity observed when pH is lowered (Table III) suggest that the protonation event itself induced a conformational change in the enzyme. Presumably, it is on this new tetramer conformation that low concentrations of urea most effectively promote formation of catalytically inactive dimers (Figs. 3 and 7).

Unlike the urea effects, the influences of the methylamines on phosphofructokinase aggregation state did not show a pH dependence. Upon addition of trimethylamine-N-oxide (400 mM), phosphofructokinase light scattering increased initially by 20–25% when measured at either pH 6.7 or 8.0. The subsequent decreases in light scattering which occurred at pH 6.7 and 6°C were correlated with changes in enzyme activity and were both reduced by the presence of trimethylamine-N-oxide.

Another factor that modulates the sensitivity of phosphofructokinase to urea perturbation and alters the degree of stability afforded the enzyme by methylamines is enzyme concentration (e.g. Fig. 4). Due to the strong protein concentration dependence of the enzyme's aggregation state (17, 31, 32), it is not unexpected that solutions which affect this aggregation would likewise exhibit such a dependence (although this type of effect is clearly not required) (see Ref. 51). Realistic estimates of intracellular phosphofructokinase concentrations are necessary to permit statements about the importance of these solute, pH, and temperature effects in vivo. In this regard, it is pertinent to emphasize that phosphofructokinase is an ambiquitous enzyme (52), i.e. an enzyme whose intracellular distribution is not fixed, but rather may alternate between soluble and particulate (bound) forms according to local microenvironmental conditions.

Metabolic Implications of pH-Temperature-Solute Interactions on Phosphofructokinase—The interactions among pH, temperature, and low concentrations of nitrogenous solutes have potential regulatory roles in vivo. In working skeletal muscle, reductions in pH could favor decreases in glycolytic flux (with concomitant prevention of further damaging pH decreases) via shifting the tetramer-dimer equilibrium in favor of inactive dimers (53, 54). Reversible inhibition of phosphofructokinase via dimer formation may also be important during various types of metabolic torpor. For example, the intracellular microenvironment of a hibernating mammal (2°C, pH 6.8–7.0, 30 mM urea) (55, 56) seems inimical to the preservation of the tetrameric state of phosphofructokinase. Suppression of glycolytic flux through reversible interconversion of active and inactive forms of phosphofructokinase thus
could be important in hibernators and in many estivating forms which also build up high intracellular urea concentrations (57, 58). This conjecture is reasonable in view of our finding that phosphofructokinase from animals commonly experiencing elevated intracellular urea displays sensitivities similar to those noted for the rabbit muscle enzyme. Finally, the pronounced stabilization of phosphofructokinase by low concentrations of methylamines suggests that these solutes may play important roles as protein structure stabilizers in methylamine-rich plants and animals (15, 22).

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REFERENCES

33. Wizor, D. J. (1969) In Physical Principles and Techniques in
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