Effect of Urea and Organic Solvents on the Mitochondrial $F_1$ ATPase

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Urea, Mitochondrial $F_1$ ATPase

Mitochondrial $F_1$ ATPase is inactivated by urea. Protection against urea inactivation is obtained when betaine, a methylamine found in different tissues, is added to the assay medium. Protection is also obtained upon the addition of either glycerol or dimethyl sulfoxide to the assay medium. The $F_1$ ATPase is rapidly inactivated at $4^\circ$C. Inactivation by low temperature is prevented by betaine, glycerol and dimethyl sulfoxide. The protective effect of organic solvents and betaine against cold inactivation is prevented by urea.

Introduction

Concentrations of urea varying from 0.4 to 0.8 M occur commonly in the mammalian kidney and throughout the tissues of cartilaginous fishes [1, 2]. In these fishes the high concentration of urea serves to balance the high osmolarity of the sea water. Under extremes of water stress, as might occur in desert rodents [3], urea concentrations in the urine may rise to above 4 M. Urea readily diffuses across cellular membranes and its concentration in cells is practically the same as that found in the extracellular fluids [1–3]. Urea is a protein denaturant and at low concentrations, less than 1 M, it may cause slight alteration of the protein structure but drastically alter its biological function [1, 2, 4–6]. At present we do not know how cells are protected from the toxic effect of urea. Recently it has been proposed that methylamines may serve to protect cells from urea by counteracting its deleterious effect on protein structure [1, 2, 5, 7]. Methylamines such as trimethylamine-N-oxide, betaine (1-carboxy-N,N,N-trimethyl-ethanaminium hydroxide inner salt) and glyceryl-phosphorylcholine have been observed in different types of tissues [2, 8–10]. Using $^{14}$N NMR, Balaban and Knepper [10] estimated that the intracellular concentration of methylamine compounds in rat and rabbit kidney is near 200 mmol/kg of intracellular water. Yancey and Somero [1] reported that the activity of arginosuccinate lyase from pig kidney or from liver of the thornback ray is inhibited by $0.2–0.4$ M urea, and that this effect is abolished when trimethylamine-N-oxide is added to the assay medium together with urea. Wilkie and Wray [7] measured the concentration of glycerylphosphorylcholine in the gastrocnemius muscles of frogs by $^{31}$P NMR spectroscopy. They observed a simultaneous increase in blood urea and in the concentration of the methylamine in muscle when frogs kept in tap water were exposed to water made hypertonic with NaCl. We observed that the phosphorylation by orthophosphate of the Ca$^{2+}$-ATPase of sarcoplasmic reticulum is inhibited by urea and that inhibition is counteracted not only by methylamine but also by different organic solvents [6]. The finding that the effect of methylamines may be duplicated by organic solvents raises the possibility that the antagonism between methylamines and urea could be related to changes in the activity of water in the medium. In this report this hypothesis was explored further with the use of soluble mitochondrial $F_1$ ATPase.

Materials and Methods

$F_1$ ATPase was prepared from bovine heart mitochondria as previously described [11]. Under the conditions used in this report, in totally aqueous medium and at $30^\circ$C, the activity of different $F_1$ preparations used varied between 57 and 65 $\mu$mol/min.mg protein. This activity is taken as 100% in the figures.

ATPase activity was assayed by measuring the release of P from ($\gamma$-$^{32}$P) ATP as previously described [12].

Results

Effects of betaine and organic solvents

The ATPase activity of $F_1$ was inhibited when either urea, glycerol or betaine was added to the assay
Fig. 1. Effect of organic solvents, betaine and urea. The assay medium composition was 50 mM Tris-Cl buffer (pH 7.5), 3 mM MgCl₂ and 3 mM (γ-P) ATP. The reaction was started by the addition of soluble F₁ to a final concentration of 2 μg protein per ml and arrested after 3 min incubation at 30 °C by the addition of an equal volume of activated charcoal suspension in 0.1 M HCl. After centrifugation a sample of the clear supernatant was counted in a scintillation counter. (○) urea, (■) dimethyl sulfoxide, (▲) glycerol and (●) betaine.

medium. At the concentrations used, dimethyl sulfoxide had no effect on the enzyme activity (Fig. 1). The inhibition produced by glycerol and betaine was reversible. The enzyme activity was fully restored after an assay mixture containing 3 M of either glycerol or betaine was diluted 30 fold with a reaction medium that did not contain organic solvent or betaine. The inhibition promoted by 1 M urea was not reversible; activity was not restored after decreasing the urea concentration from 1 M to 0.03 M (data not shown).

Dimethyl sulfoxide, glycerol and betaine protect the enzyme from urea. This could be more clearly observed with the use of dimethyl sulfoxide. Up to a concentration of 3 M, dimethyl sulfoxide had no effect on the enzyme activity (Fig. 1). However, in the presence of this solvent the concentration of urea needed to promote a 50% inhibition of the ATPase was higher than that needed in totally aqueous medium (Fig. 2A). A similar effect was observed with the use of betaine (Fig. 2B). With this compound there was a mutually opposing effect; i.e. betaine antagonized the inhibitory effect of urea and conversely, urea antagonized the inhibitory effect of betaine. In absence of urea, the addition of 1 M betaine to the medium promoted a 60% inhibition of the ATPase activity (Fig. 2B). Addition of increasing concentrations of urea to the media containing 1 M betaine did not further inhibit the enzyme, but on the contrary, led to an increase in the ATPase activity until the concentration of urea was the same as betaine. In presence of 1 M of both betaine and urea, the ATPase activity was about 82% of that measured in the absence of both drugs. Activity declined when the concentration of urea exceeded that of betaine. There was a 90% inhibition of ATPase activity in the presence of 2 M betaine. In this case, addition of urea led to an increase in activity and there was no further inhibition even after raising the urea concentration to 3 M (Fig. 2B). A similar mutually opposing effect was observed when urea and glycerol were used (Fig. 2C). Glycerol was less effective than betaine, both as an inhibitor of the F₁ ATPase activity and as an antagonist of the deleterious effect of urea.

Effect of temperature

The soluble F₁ ATPase is readily inactivated when incubated at 4 °C (Fig. 3). Penefsky and Warner [13] observed that glycerol protects then enzyme from cold-inactivation. We observed that in addition to glycerol (Fig. 3C), betaine (Fig. 3B) and dimethyl
sulfoxide (Fig. 3A) were also able to protect the $F_1$ from cold inactivation. The protection exerted by these three compounds was antagonized by increasing concentrations of urea (Fig. 4).

**Discussion**

At present we do not know the structure of water in the cell. Muscle cells contain approximately 23% protein by weight; red blood cells contain about 35% protein by weight; and in general actively growing cells contain between 17% and 26% protein by weight [14]. Because of these high protein concentrations, it is unlikely that in physiological conditions the solvent structure in the cytosol is the same as that of the dilute aqueous solutions usually used for “in vitro” experiments [14—16]. The water molecules that organize around a protein in solution have properties that are different from those of medium bulk water, for example a lower vapor pressure, a lower water mobility and a greatly reduced freezing point [15, 16]. Similar changes in the properties of water are observed in mixtures of organic solvents and water [17—19]. Kundu and Das [20] reported that urea and organic solvents such as dimethyl sulfoxide have opposing effects on the interactions between anions and water. These data may indicate that under physiological conditions high urea concentrations are not deleterious to the cell due to the changes in water structure promoted by proteins and other organic polymers found in the cell. The finding that betaine protects the Ca$^{2+}$-ATPase [6] and the $F_1$ ATPase from the toxic effect of urea in a manner similar to that observed with organic solvents may indicate that methylamines such as betaine, by promoting changes in water structure, can contribute to protection of the cell from the deleterious effect of urea. According to this hypothesis, in the cell proteins as well as the different methylamines would modify the properties of bulk water in a manner similar to that observed with the use of organic solvents, and urea would antagonize this effect. Finally, it should be mentioned that in Figs 1 to 4 we used concentrations of urea and betaine higher than those found in physiological condition.

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