ELECTROGENIC BICARBONATE ION TRANSPORT THROUGH THE INNER MITOCHONDRIAL MEMBRANE INDUCED BY CYTOPLASMIC GLYCOPEPTIDE

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By experiments with isolated mitochondria (MC) a number of transport systems for anions and metabolites have been identified in their inner membrane [3, 7]. Nevertheless, the data so far obtained have not answered the question of how activity of the transport systems of the inner mitochondrial membrane (IMM) is regulated by hormones and mediators of the nervous system *in vivo*. It is now evident that hormonal regulation of mitochondrial function is effected through intracellular intermediaries of hormones [4, 5, 9, 13].

The writers showed previously that in hyperthyroidism activity of a thermostable factor, which uncouples oxidative phosphorylation and increases permeability of the IMM for phosphate ions [2], is increased in cytosol of rat liver and heart. This factor has been isolated and identified as a glycopeptide with mol. wt. of about 3 kilodaltons (kD) [1].

The present investigation shows that the cytoplasmic glycopeptide induces pH-dependent electrogenic bicarbonate ion transport through the IMM.

EXPERIMENTAL METHOD

MC were isolated from the liver of noninbred male rats weighing 120-140 g in medium containing 0.3 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.5 [8]. The cytoplasmic glycopeptide was isolated from the liver of satiated hyperthyroid male rats weighing 90-100 g by the method described previously [1] and was desalinified on a column with Sephadex G-10 (20 × 700 mm), and eluted with bidistilled water. Hyperthyroidism was induced by giving the rats thyroxine in a dose of 1 mg/kg daily for 4 days. The kinetics of swelling of MC was studied by measuring changes in optical density of MC on an LMF-69 photometer. MC (1.4 mg protein) were preincubated for 2 min at 25°C in the presence of cytoplasmic glycopeptide in 0.5 ml of medium containing 0.12 M KCl, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.6. The suspension of MC was treated with $2 \cdot 10^{-5}$ M 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (acetazolamide). Swelling of MC was initiated by the addition of 3.0 ml of incubation medium containing 0.12 M $KHCO_3$, 1 mM EDTA, 10 mM Tris-HCl, 0.7 µg/ml rotenone, 0.6 µg/ml valinomycin, and $2 \cdot 10^{-5}$ M acetazolamide. A generally accepted test for measuring permeability of the IMM for anions is to measure the kinetics of swelling of de-energized MC in iso-osmotic solution of potassium salts of the test anions in the presence of valinomycin, when the rate of swelling of MC is limited by transport of the anions through the IMM [6]. This test was used by the present writers to study the action of cytoplasmic glycopeptide on bicarbonate ion transport through the IMM. Experiments were carried out in the presence of acetazolamide ($2 \cdot 10^{-5}$ M) [12], a carbonic anhydrase inhibitor, which prevented accumulation of HCO_3^- ions in the matrix of MC, a process which is connected with diffusion of CO_2 into MC.

It will be clear from Fig. 1A that the initial rate of swelling of de-energized rat liver MC in iso-osmotic $KHCO_3$ solution in the presence of valinomycin and acetazolamide was very low. Consequently, the IMM has low permeability for HCO_3^- ions through the IMM. If acetazolamide was omitted from the mitochondrial incubation medium the rate of glycopeptide-induced swelling of MC was sharply reduced, although the basal rate of swelling of MC was increased (Fig. 1B). In the absence of acetazolamide, HCO_3^- ions are evidently partially converted into CO_2 in the carbonic anhydrase reaction and, as a result, the concentration of

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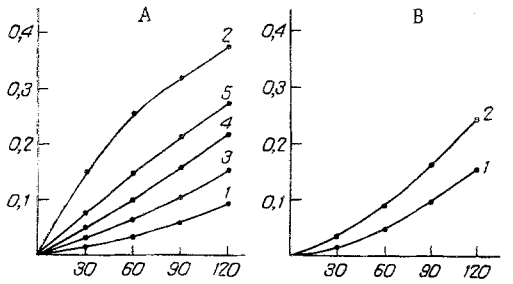

Fig. 1. Swelling of de-energized rat liver MC in iso-osmotic solution of $KHCO_3$ in presence of valinomycin. Abscissa, time (in sec); ordinate, optical density at 540 nm (in relative units). A) Preincubation medium and incubation medium with acetazolamide ($2 \cdot 10^{-5}$ M); B) preincubation medium and incubation medium without acetazolamide. 1) Without cytoplasmic glycopeptide; 2) cytoplasmic glycopeptide (3 μg protein/ml); 3) preincubation medium + 10^{-4} M DNP + cytoplasmic glycopeptide (3 μg protein/ml); 4) preincubation medium + antimycin (1 μg/ml) + cytoplasmic glycopeptide (3 μg protein/ml); 5) preincubation medium + rotenone (1 μg/ml) + cytoplasmic glycopeptide (3 μg/protein/ml).

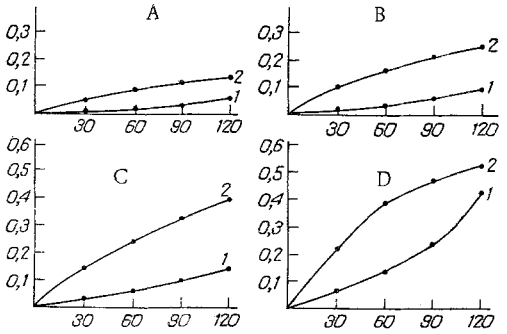

Fig. 2. Dependence of swelling of de-energized rat liver MC in iso-osmotic $KHCO_3$ solution in presence of valinomycin on pH of incubation medium. A) pH 7.0, B) pH 7.6, C) pH 8.1, D) pH 8.9. 1) Without cytoplasmic glycopeptide, 2) cytoplasmic glycopeptide (3 μg protein/ml). Remainder of legend the same as to Fig. 1.

HCO_3^- ions in the mitochondrial matrix is reduced. It is well known that the IMM has very low permeability for H^+ or OH^- ions [3]. In the absence of an uncoupler of oxidative phosphorylation in the incubation medium the rate of swelling of MC in iso-osmotic solutions of potassium salts in the presence of valinomycin is therefore determined by the kinetics of electrogenic transport of anions [6]. Electroneutral symport of anions with H^+ ions in this model system does not take place in the absence of an uncoupler of oxidative phosphorylation, for transfer of charges and H^+ ions through the IMM is not compensated in this case [6]. The action of cytoplasmic glycopeptide on swelling of MC in iso-osmotic $KHCO_3$ solution was manifested in the absence of 2,4-dinitrophenol (DNP) in the incubation medium. Consequently, cytoplasmic glycopeptide induces electrogenic transport of HCO_3^- ions across the IMM but not electroneutral transport of bicarbonate ions in symport with H^+ ions.

We know that transport of anions such as NO_3^- and Cl^- through the rat liver IMM takes place through a pH-dependent pore for anions [10, 11]. It might be supposed that transport of HCO_3^- ions induced by cytoplasmic glycopeptide is effected through a pH-dependent pore in the IMM also. Dependence of the rate of swelling of MC in iso-osmotic $KHCO_3$ solution on the pH of the incubation medium is illustrated in Fig. 2. With an increase in pH of the incubation medium within the range from 7.0 to 8.9 the basal rate of swelling of de-energized MC in iso-osmotic $KHCO_3$ solution in the presence of valinomycin and acetazolamide increases sharply. Meanwhile, with an increase in pH of the incubation medium between 7.0 and 8.0 the

action of cytoplasmic glycopeptide on transport of HCO_3^- ions through the IMM is intensified. A further increase in pH of the incubation medium increased the basal permeability of the IMM for HCO_3^- ions, but reduced the cytoplasmic glycopeptide-dependent component of transport (Fig. 2). A similar increase in permeability of IMM for NO_3^- and Cl^- ions with an increase in pH of the incubation medium has been described in the literature [10, 11].

The experimental results are evidence that, like transport of NO_3^- and Cl^- ions, electrogenic transport of HCO_3^- ions through the IMM is effected by a pH-dependent anionic pore; functioning of this pore, moreover, is controlled by cytoplasmic glycopeptide. To make this action of cytoplasmic glycopeptide on HCO_3^- ion transport manifest, the MC must be preincubated in its presence, under conditions of energization of the organelles. Without preincubation, the effect of stimulation of HCO_3^- transport does not appear. Addition of respiration inhibitors or of 2,4-DNP to the preincubation medium of MC abolished the action of the glycopeptide on HCO_3^- ion transport (Fig. 1A). Meanwhile inhibitors of respiration and 2,4-DNP do not affect glycopeptide-induced bicarbonate ion transport.

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MEMBRANE-BOUND Ca^{2+} IN MITOCHONDRIA OF EHRLICH'S ASCITES TUMOR CELLS

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Ehrlich's ascites tumor cells, like cells of many other malignant tumors, are characterized by significant changes in energy metabolism [13] and the Ca^{2+} transport system [10]. Investigations have shown that changes in the Ca^{2+} transport system are the primary causes of changes observed in energy metabolism of tumor cells [6, 7]. We know, for instance, that mitochondria (MC) of tumor cells can assimilate and retain unusually high quantities of Ca^{2+} and that even low concentrations of this ion inhibit their oxidative phosphorylation [2, 7, 15].

The concentration of bivalent ions in MC is an important parameter determining their functional activity [11]. However, insufficient attention has so far been paid to the study of the mechanisms regulating the free Ca^{2+} level in MC.

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