REGULATION OF PERMEABILITY OF THE MITOCHONDRIAL MEMBRANE BY MITOCHONDRIAL INTERACTION FACTOR

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The effect of mitochondrial interaction factor (MIF) on swelling of the mitochondria in isoosmotic media with KNO_3, $Ca(NO_3)_2$, and NH_4NO_3 was studied in the presence of oxidation inhibitors. The total MIF fraction was shown to inhibit swelling of the mitochondria in all three media. By elution of MIF from a DEAE-cellulose column with a pH gradient falling from 8.7 to 6.7, three peaks of activity were obtained (fractions A, B, and C). Quantitative differences were found in the action of these three fractions on swelling of the mitochondria. Fraction C inhibited swelling more strongly in $Ca(NO_3)_2$, whereas fractions A and B did so in media with KNO_3 and NH_4NO_3.

KEY WORDS: mitochondria; swelling; mitochondrial interaction factor; regulation of ion transport.

In the cell, mitochondria are fairly heterogeneous, and the heterogeneity of the mitochondrial population increases when the tissue is under favorable conditions [8, 9]. A previous investigation [2] showed that mitochondria if "damaged" by thyroxine or Ca^{++} ions, which have completely lost their ability to carry out oxidative phosphorylation and active ion transport, can affect the function of intact mitochondria. It was shown [2] that this effect is due to a low-molecular-weight factor liberated from mitochondria under conditions of "high amplitude swelling" and capable of modifying the parameters of respiration and active Ca^{++} transport in intact mitochondria.

This paper describes a study of the effect of mitrochondrial interaction factor (MIF) on the permeability of the mitochondrial membrane to K^+, H^+, and Ca^{++} ions.

EXPERIMENTAL METHOD

Mitochondria were isolated from the albino rat liver by Schneider's method [6]. Swelling of the mitochondria was assessed from changes in optical density at 520 nm. MIF was isolated by the method described earlier [2] but, instead of successive gel filtrations on Sephadexes G-50 and G-10, gel filtrations were carried out on a Sephadex G-25 column measuring 95×2.5 cm. After equilibration, elution was carried out with distilled water. Fractions containing activity (low-molecular-weight yellow peak) were collected and applied to a DEAE-cellulose column (15×300 mm) equilibrated with 5 mM tris-HCl, pH 8.7. The MIF was eluted from the column by 100 ml of 5 mM tris-HCl, pH 6.7.

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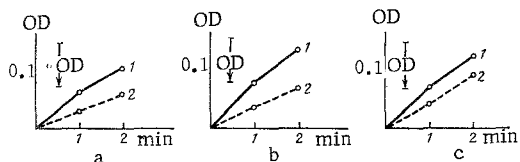

Fig. 1

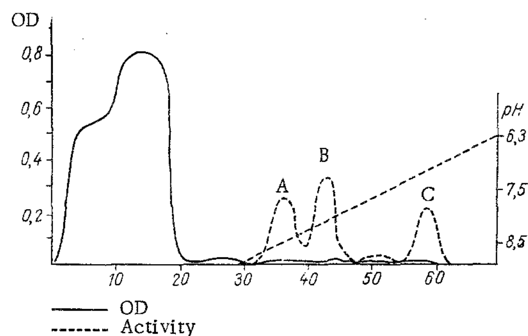

Fig. 2

Fig. 1. Effect of MIF on swelling of mitochondria. Mitochondria were added to incubation medium in an amount of 0.45 mg protein to 1 ml; the medium contained: a) 0.1 M KNO_3, 0.01 M tris, pH 6.45, rotenone 0.5 μg/ml; b) 0.1 M NH_4NO_3, 0.01 M tris, pH 6.45, rotenone 0.5 μg/ml; c) 0.015 M $Ca(NO_3)_2$, 0.1 M tris, pH 6.45. MIF was added in a volume of 0.2 ml to a 3-ml cell. OD) Optical density; 1) control; 2) addition of MIF.

Fig. 2. Fractionation of MIF on DEAE-cellulose column. Abscissa, No. of 4-ml samples; ordinate, left, OD at 280 nm; right, pH gradient (curves with 3 peaks relative to activity on mitochondrial swelling).

EXPERIMENTAL RESULTS

Maximal effects of MIF as a rule were exhibited at pH values below 7.0 and on mitochondrial preparations which had aged for a few hours in the cold. Under these conditions one cause of "uncoupling" of the mitochondria is the permeability of the mitochondrial membrane to K^+ and H^+ ions. The action of MIF can possibly be explained by inhibition of the permeability of the mitochondrial membrane to K^+ and H^+ and a corresponding increase in the respiratory control.

The inner mitochondrial membrane is readily permeable to the NO_3^- anion. When the mitochondria were placed in a 0.1 M KNO_3 + 0.01 M tris in the presence of rotenone, NO_3^- and K^+ ions (if the membrane was permeable to potassium) and, correspondingly, water entered the internal space of the mitochondria along the concentration gradient. Under these conditions the rate of swelling of the mitochondria was determined by the permeability of the membrane to K^+, for permeability to NO_3^- is very high and mitochondria have the properties of an ideal osmometer. On addition of MIF to the incubation medium of the mitochondria, the initial rate of swelling was inhibited in medium with KNO_3 (Fig. 1a). This effect also was observed when another penetrating anion, such as phosphate ions, was present instead of NO_3^- in the incubation medium. MIF evidently reduced the permeability of the mitochondrial membrane to K^+ ions.

MIF also had a similar action on swelling of the mitochondria in media with $Ca(NO_3)_2$ in the presence of rotenone; if the $Ca(NO_3)_2$ concentration was lowered but the isotonicity of the medium preserved, the degree of inhibition was increased. This is evidence of the competitive character of inhibition of membrane permeability for Ca^{++} ions (Fig. 1c).

Permeability of the mitochondrial membrane to H^+ ions can be judged from the rate of swelling in media with NH_4NO_3 [1]. The mitochondrial membrane is readily permeable to NO_3^- ions and NH_3 but not to NH_4^+. As a result of the reaction $NH_3 + H_2O \rightarrow NH_4^+ + OH^-$, OH^- ions accumulate in the mitochondrial matrix, and it is this alkalification of the mitochondrial matrix which prevents swelling of the mitochondria in media with NH_4NO_3. Nevertheless, if the permeability of the mitochondria to H^+ ions is sufficiently great, mitochondria will swell rapidly in media with NH_4NO_3. This can be observed on the addition of an uncoupling agent, during aging of a mitochondrial suspension, and under conditions pathological for the tissue. Experiments were carried out on mitochondrial preparations which had aged for a few hours in the cold at pH 6.5, and for that reason their membrane was relatively readily permeable to H^+ ions, and they swelled in medium with 0.1 M NH_4NO_3. This type of swelling of the mitochondria (Fig. 1b) was also inhibited by MIF. The results of these experiments thus confirmed the hypothesis expressed above, namely that the action of MIF on respiration and on the transport of Ca^{++} ions in the mitochondria can be explained by a decrease in their permeability to K^+ and H^+ ions.

The action of MIF on permeability of the membrane to Ca^{++} ions requires further explanation. The writers' previous investigation showed that MIF not only does not inhibit, but actually slightly increases the rate of transport of Ca^{++} ions in mitochondria [2]. The mechanism of active transport of Ca^{++} ions in mitochondria has not yet been explained. The electrophoretic scheme of active ion transport in mitochondria suggested by Mitchell readily explains the character of transport of K^+ ions in the presence of valinomycin and some synthetic cations, but does not explain the phenomenology of Ca^{++} ions transport in mitochondria [4]. For instance, an increase in the permeability of the mitochondrial membrane by the addition of Ca^{++} ionophores increase the outflow of Ca^{++} from the mitochondria and inhibits the active transport of Ca^{++} ions into them [7]. The addition of ruthenium red to mitochondria in concentrating inhibiting the active uptake of Ca^{++} by mitochondria causes an outflow of Ca^{++} accumulated previously from the mitochondria. Evidently besides active Ca^{++} transport, a passive outflow of Ca^{++} also takes place from mitochondria along the concentration gradient; these two processes, moreover, differ in their sensitivity to inhibitors of Ca^{++} transport. This point of view has been expressed previously [3]. On the basis of this explanation, the inhibition of passive Ca^{++} transport in mitochondria by MIF, if active Ca^{++} transport in them is insensitive to MIF, ought to lead to an increase in the ability of the mitochondria to maintain the gradient of Ca^{++} ions on their membrane.

By eluting MIF from a DEAE-cellulose column with a falling pH gradient from 8.7 to 6.7, not one but three peaks of activity of MIF were obtained (Fig. 2). The fractions corresponding to these peaks were collected and their action on swelling of mitochondria was tested in media containing nitrates of Ca^{++}, K^+, and NH_4^+. The tests showed that fraction A (first peak of activity) inhibited swelling considerably in medium with KNO_3, its effect was much weaker in medium with NH_4NO_3, and it had hardly any effect on swelling in medium with Ca $(NO_3)_2$. The fraction B (second peak) inhibited swelling of the mitochondria in medium with NH_4NO_3 and KNO_3 and inhibited swelling only weakly in medium with $Ca(NO_3)_2$. The fraction C (third peak) inhibited swelling in medium with $Ca(NO_3)_2$. By analogy with the experiments described above, these results can be interpreted as follows: Fraction A affects permeability of the mitochondrial membrane for K^+ ions, fraction B affects permeability for H^+ and K^+ ions, and fraction C affects permeability for Ca^{++} ions. The mechanism of action of these three fractions of MIF evidently differs qualitatively.

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