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EFFECT OF DITHIOTHREITOL ON TISSUE-SPECIFIC UNCOUPLING OF OXIDATIVE PHOSPHORYLATION BY RAT LIVER MITOCHONDRIA

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The present writers have shown that the liver [4] as well as the kidney, heart, lungs, and thymus of rats [3] contain regulators which can selectively depress the coupling of oxidative phosphorylation (OP) by mitochondria from homologous tissue. A study of the properties of a tissue-specific uncoupler (TSU) from the soluble phase of liver cells showed that this regulator is a low-molecular-weight compound with mol. wt. under 5000, and with a negative charge at alkaline pH values. A combination of gel filtration with ion-exchange chromatography on DEAE-cellulose enabled it to be purified about 200 times relative to protein [6]. However, further fractionation of the TSU was much more difficult because of its inactivation during purification. The necessity thus arises for a study of the mechanism of this process. One of the most widespread causes of inactivation of bioorganic compounds is autooxidation of the sulfhydryl groups which they contain [1]. In some cases it is reversible and the activity of some compounds can be restored by simultaneous incubation with thiol compounds, such as dithiothreitol (DTT) [7].

No data could be found on the existence of active SH-groups in the regulator which we discovered. Experiments were therefore carried out to study the action of DTT on the activity of the TSU, which had been lost in the course of the preparative procedures, with a view to establishing whether the regulator may contain sulfhydryl groups.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-250 g. The fraction of mitochondria from liver and kidney was isolated by Schneider's method with modifications. The soluble phase of liver cells was obtained by simultaneous sedimentation of all organelles from the homogenate by ultracentrifugation at 105,000g (1 h). Activity of TSU in this fraction was determined polarographically by measuring the selective increase in uptake of oxygen in the active metabolic state by liver mitochondria (ΔO_{act}). Data on the effect of the test fraction on the corresponding parameter of kidney mitochondria served as the control. The soluble phase of the cells was subjected to gel filtration on a column with Sephadex G-25 [6]. By means of this procedure the regulator could be purified 15-16-fold relative to protein. Thermal fractionation was carried out by heating the soluble phase to 90°C for 10 min. The denatured proteins were removed by centrifugation. DTT used in the experiment was from Calbiochem. Incubation with DTT continued for 1 h at 32°C. The results given in the paper are average results of five experiments.

EXPERIMENTAL RESULTS

To begin with, it was established that addition of DTT in a concentration of 10 mM to intact liver and kidney mitochondria reduced the value of ΔO_{act} of these mitochondria by 8-

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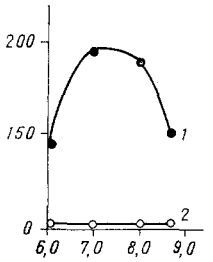

Fig. 1

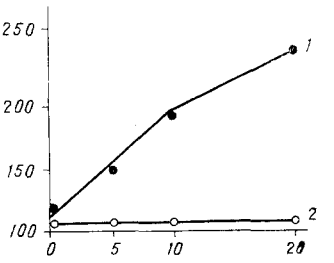

Fig. 2

Fig. 1. Dependence of action of DTT on TSU activity in liver (1) and (2) kidney mitochondria on pH of reaction medium. Abscissa — pH; ordinate — ΔO_{act} (in % of their values in intact mitochondria in the presence of 10 mM DTT). Initial activity of TSU before heating soluble phase was 210 ± 8%.

Fig. 2. Dependence of activating action of DTT on TSU on concentration of reagent. Abscissa — DTT concentration (in mM). Remainder of legend as in Fig. 1. Conditions of obtaining TSU-containing fraction the same as in Fig. 1. Initial activity of TSU before heating was 225 ± 6%. Incubation with DTT carried out at pH 8.0; volume of samples equalized by addition of corresponding volumes of bidistilled water.

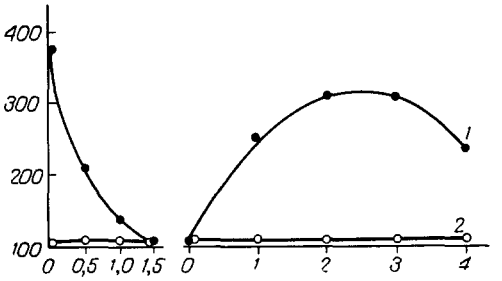

Fig. 3. Effect of DTT (20 mM) on activity of TSU lost during incubation of the previously heated soluble phase of the cells. Abscissa — time (in h); gap corresponds to keeping fraction with inactivated TSU at —25°C. Remainder of legend as in Fig. 1.

10%, consequently, this reagent has no significant effect on the coupling of OP. The TSU-containing fraction obtained after gel filtration on Sephadex G-25 (coarse), with an initial activity (as ΔO_{act} in % of intact mitochondria) of 294%, was then incubated with DTT. As a result of keeping at pH 9.2 and at —25°C for 3 days, activity fell to 172%. This fraction was then incubated with 20 mM DTT at pH 8.5 for 1 h and activity of the TSU was measured. After incubation it increased to 320%, indicating that DTT can restore activity of TSU lost in the course of keeping of the partially purified fraction. DTT has no effect on the tissue-specificity of action of TSU.

It was necessary to determine optimal conditions for activation of TSU under the influence of DTT. For this purpose the effect of the pH of the reaction medium on activation was studied (Fig. 1). In these and in all subsequent experiments a TSU-containing fraction obtained by heating the soluble cell fraction was used. Only fractions which had completely lost their TSU activity after this procedure were chosen. Activation of TSU in the presence of DTT had a well-marked pH-optimum within the range pH 7.0-8.0, but the process we were studying could also be observed at pH values of 6.0 and 8.5. DTT had no effect on the tissue specificity of action of TSU at any pH value tested. Dependence of activation of TSU on the DTT concentration in the incubation medium also was investigated (Fig. 2). With an increase in the concentration of this reagent to 20 mM activity of TSU increased; up to a concentration of 10 mM this increase was linear in character. The tissue specificity of action of TSU was not reduced by DTT in any of the concentrations tested. It was shown previously that in the course of keeping of TSU at a pH of under 9.0, this regulator was inactivated even at —25°C [6]. An attempt was made to restore, by means of DTT, the activity of TSU which it had lost during incubation at neutral pH values (Fig. 3). Fractions in which TSU activity was not reduced af-

ter heating were used in these experiments. These fractions were incubated at pH 7.0 (37°C) until total inactivation of the TSU, after which they were kept for 24 h at −25°C and then incubated with DTT. It was found that TSU activity was restored almost to its initial level as early as 2 h after the beginning of incubation. It remained unchanged until 3 h, and then declined a little until 4 h after the beginning of incubation with DTT.

The results are evidence that DTT can reactivate TSU which has lost its activity under the influence of various factors. This reagent proved equally effective when acting on fractions inactivated by heating or during incubation at alkaline pH (−25°C). Incubation of TSU with DTT enabled the activity of the regulator to be restored when inactivated by incubation at pH 7.0 (37°C). The results thus demonstrate that TSU contains active SH-groups. Autooxidation of these groups was found to be the cause of inactivation of the regulator in all the cases mentioned above. For this reason the action of TSU on OP in the mitochondria can be regarded as a mechanism ensuring the optimal level of OP in the living cell. Under the influence of TSU, tissue-specific inhibition of mitochondrial OP takes place, leading to a reduction in the intensity of intracellular reduction processes. Once a definite level of oxidation is reached in the cell, TSU may therefore be inactivated by oxidation of its SH-groups by a negative feedback principle.

The writers previously examined the possible role of TSU as an effector of internal regulation of proliferation in the tissues [2]. TSU may have an inhibitory action on mitotic activity by two fundamentally different mechanisms. It inhibits mitosis in cells which have already started the cycle, by inhibiting endergonic processes taking place in them. In cells in interphase, this regulator is responsible for switching the genome from the "mitotic cycle program" to the "resting state program," in which the cell performs a special tissue function. The first program is linked with intracellular DNA synthesis. In the course of realization of the second stage this synthesis is inhibited, whereas protein synthesis increases sharply. Meanwhile, TSU brings about exhaustion of the stores of metabolites which are precursors for DNA synthesis in these cells and accumulation of others needed for protein synthesis. This is achieved by a fall in the ATP level and by stimulation of respiration during uncoupling of mitochondrial OP through the action of this regulator. According to Jacob and Monod's scheme, metabolites are inducers of the enzymes responsible for their conversion [8]. During uncoupling of mitochondrial OP in cells in interphase under the influence of TSU, repression of the "mitotic cycle program" operons must be expected with activation of "resting state program" operons.

For the mechanism described above to be realized it is important that the TSU concentration in the cells increases proportionally to mitotic activity of the tissue. This could happen if cells in mitosis produced TSU. We have demonstrated that TSU is activated when the water-soluble fraction of the cell nucleus is incubated together with the soluble phase of cytoplasm. This process has been shown to be sensitive to the ionic strength and pH of the reaction medium and also to the presence of bivalent cations in it [5]. Since the nuclear membrane is completed degraded in the prophase of mitosis and the contents of the nucleus come into contact with the cytoplasm, activation of TSU must be expected in the mitotic cell. This view is confirmed by data in the literature on changes in energy metabolism of the cell during mitosis, which we have discussed previously [4]. The information obtained on the existence of active SH-groups capable of reversible oxidation, in TSU suggests that intracellular activation of this regulator may take place through their enzymic reduction. These observations also point to the important role of SH-groups in the regulation of mitotic activity of tissues.

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