RESPIRATION-STIMULATED ACTIVATION OF DEOXYRIBONUCLEASE I ASSOCIATED WITH MITOCHONDRIA

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The effect of respiration and calcium transport in the liver mitochondria on activation of DNase I associated with the organelles was investigated. It was shown that 96% of the total activity of this enzyme in the mitochondria is in a latent state. Aeration of a suspension of mitochondria leads to a sharp rise in its activity. Activation of DNase I is inhibited by the addition of EGTA and stimulated in mitochondria releasing calcium. The pH optimum of EGTA-inhibited activation of DNase I was shown to be 8.0. It is concluded that activation of this enzyme depends on the state of cellular energetics. It is suggested that a role is played by mitochondrial phospholipase A, activated during release of calcium from the mitochondria in the process of activation of DNase I.

KEY WORDS: Mitochondria; DNase I; calcium; respiration; phospholipase A.

DNase I is one of those enzymes whose activity rises sharply after injury to the tissues of a living organism. An increase in its activity has been observed in radiosensitive tissues of irradiated animals [4] and in the regenerating liver [12]. However, the process of activation of DNase I has not yet been investigated. More than half of the total activity of this enzyme in rat liver cells is known to be concentrated in the fraction of mitochondria (MCH) [10]. This localization of the enzyme is difficult to explain, for less than 1% of the total cell DNA is contained in MCH.

It might be supposed that association of DNase I with MCH is due to the manner of regulation of its activity in the cell. In this case the existence of correlation would be expected between DNase activity in MCH and the course of intramitochondrial processes.

The object of the present investigation was to test the writers' previous hypothesis concerning activation of DNase I during release of Ca^{++} from the matrix of MCH [6].

METHODS

The MCH fraction was obtained from the liver of Wistar rats weighing 250-300 g by Schneider's method with modification. The state of the MCH preparation was assessed before incubation by investigation of oxidative phosphorylation by a polarographic method under the conditions described previously [9]. The calcium capacity of MCH was determined by a pH-metric method based on exchange of Ca^{++} for protons [3]. MCH with an ADP : O ratio on succinate of not less than 1.7 and with a respiratory control (after Chance) of not less than 2.5 were used in these experiments. The calcium capacity of these preparations varied from 25 to 35 μg $CaCl_2$/mg MCH protein. The suspension of MCH was incubated (at 37°C) in medium containing 125 mM sucrose, 70 mM KCl, 2 mM KH_2PO_4, 20 mM Tris-HCl, pH 8.0, 0.5% caprylic alcohol to prevent frothing of the protein, 5 mM succinate, and 4 mg/ml MCH protin. The suspension was aerated by bubbling air through it at the rate of 70 ± 3 cm^3/min per 3-ml sample.

After incubation, high-polymer DNA from calf thymus (0.75 mg/ml) and 5 mM $MgCl_2$ were added to the samples for determination of DNase activity without any change in the concentrations of the other ingredient of the incubation medium. The reaction was stopped 20 min after the beginning of incubation at 37°C, pH 7.6, by adjusting the concentration of the samples to 0.5 N with cold perchloric acid.

Maximal activity was determined in the course of incubation of MCH in incubation medium

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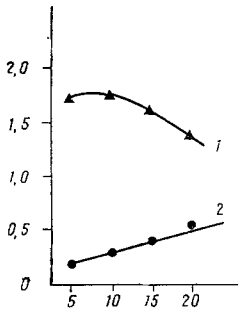

Fig. 1

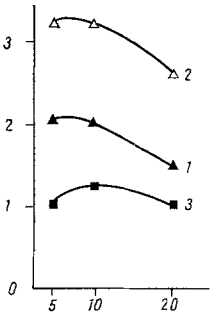

Fig. 2

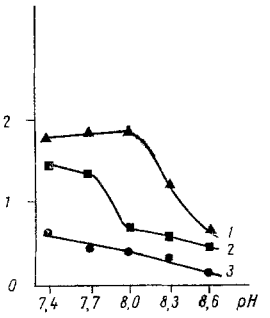

Fig. 3

Fig. 1. Dynamics of DNase I activity in suspension of aerated and unaerated MCH. Abscissa, incubation time (in min); ordinate, DNase I activity (in μg/min·mg MCH protein). 1) Aerated, 2) unaerated MCH.

Fig. 2. Effect of addition of EGTA (2 mM) and $CaCl_2$ in minimal quantity necessary to release calcium from matrix of MCH on DNase I activity in aerated suspension of MCH. 1) Aeration without addition; 2) addition of $CaCl_2$; 3) addition of EGTA. Remainder of legend as in Fig. 1.

Fig. 3. Effect of pH of incubation medium on DNase I activity after incubation of MCH under different conditions. 1) DNase I activity in aerated suspension of MCH; 2) the same, in the presence of 2 mM EGTA; 3) MCH without aeration. Remainder of legend as in Fig. 1.

to which 0.1% Triton X-100 was added, with the same conditions of exposure. Activity of DNase I was measured by staining the acid-soluble fraction by Burton's method [1]. Any special features of individual experiments will be mentioned in the text.

EXPERIMENTAL RESULTS

Initially DNase I activity was compared in intact liver MCH and in MCH disintegrated by detergent. This activity was found to be 25 times higher in the latter than in the former; consequently, DNase I in intact MCH is in a latent state. In the next experiments the effect of aeration of a suspension of liver MCH on the activity of DNase I associated with them was investigated (Fig. 1). Samples without aeration served as the control. It was shown that incubation of the unaerated suspension led to a small linear increase in DNase activity with time. Yet aeration for only 5 min led to a sevenfold increase in activity compared with the corresponding control sample. This increase still remained after aeration for 10 min. Later the DNase I activity began to fall, but even after incubation for 20 min it was still about 3 times higher than its value in the unaerated MCH suspension.

With these results in mind it was possible to study the role of Ca^{++} in the activation of DNase I in respiring MCH. For this purpose, DNase activity was compared in an aerated suspension without additives and in samples containing the chelating agent EGTA, which selectively binds Ca^{++}. In parallel experiments an MCH suspension, to which $CaCl_2$ was added in the smallest quantity necessary to cause calcium release, was aerated. The results are given in Fig. 2. Addition of EGTA was shown to cause profound inhibition of DNase I activity at all times of incubation compared with samples without additions. It was also found that the activity of this enzyme in MCH to which calcium was added was twice as high as that in the control without additions; the difference remained unchanged, moreover, until the end of the experiment.

The effect of EGTA and $CaCl_2$ on DNase I activity in a preparation of MCH previously disintegrated by the addition of Triton X-100 also was studied. Addition of EGTA in a concentration of 1-2 mM, like the addition of Ca^{++} in concentration of 1, 2, and 3 mM, was found not to affect DNase I activity under these conditions. In these experiments the important role of Ca^{++} was thus demonstrated in respiration-stimulated activation of DNase I associated with MCH.

All the previous experiments were carried out in an incubation medium with a pH of 8.0.

It was interesting to study the dependence of EGTA-inhibited DNase I activation on the pH of the incubation medium. With a rise in pH from 7.4 to 8.6, the DNase activity in the unaerated MCH suspension decreased in a linear manner. In aerated samples its activity was higher than the corresponding unaerated values over the whole range of pH values tested; maximal DNase I activity was observed in the pH range from 7.4 to 8.0, after which it fell sharply with an increase in pH. Values of DNase activity in samples with the addition of EGTA lay between its values in the experimental variants examined above (Fig. 3). Maximal activity in that case was recorded at pH 7.4-7.7; later it fell sharply at pH 8.0, and then only a little more with a further increase in pH. A marked optimum of EGTA-inhibited activation of DNase I was thus found at pH 8.0.

It was thus established that DNase I associated with MCH is present in these organelles in a latent state. Its activation is connected with disturbance of the integrity of the MCH membrane. It was shown that activation of DNase I can be produced not only by addition of detergent, but also by aeration of the MCH suspension. This effect cannot be explained by denaturation of the MCH protein as a result of frothing in the course of aeration of the suspension, for it was completely abolished by the addition of caprylic alcohol. Caprylic alcohol itself could not make any contribution to this effect, for special experiments showed that it does not affect oxidative phosphorylation of MCH in a concentration twice as high as that which was used.

In the course of incubation of the MCH suspension Ca^{++} is released spontaneously from their matrix and leads to irreversible damage to oxidative phosphorylation [2]. It has also been shown that disturbance of the function of MCH can be prevented by the addition of EDTA to the incubation medium [2]. It is generally known that in the presence of oxygen and an oxidation substrate, calcium is transported into the intramitochondrial space, until the calcium capacity is completely exhausted, after which it is released from the matrix. Addition of calcium to MCH in a quantity sufficient to cause the release of this ion from MCH was shown to stimulate activation of DNase I sharply. Calcium release is known to be accompanied by activation of mitochondrial phospholipase A, which degrades the MCH membrane [11]. It can tentatively be suggested that the action of this enzyme on MCH may activate DNAse I, like the action of a detergent. This suggestion is confirmed by the coincidence of the pH optimum of EGTA-inhibited activation of DNase I with the corresponding optimum for mitochondrial phospholipase A, which also is 8.0 [13]. The fact that activation of DNase I depends on calcium transport in MCH, which was established by these experiments, thus indicates that the increase in the activity of this enzyme was connected with disturbance of oxidative phosphorylation of MCH, for the calcium capacity falls when the functions of MCH are disturbed [2].

The dependence of activation of DNase I on disturbance of the cellular energetics is in good agreement with the fact discovered in a previous investigation, that activity of deoxyribonucleoprotein (DNP) solubilizing enzyme from the cell nucleus is inhibited in the presence of ATP [8]. This dependence confirms the hypothesis that we expressed previously, that the enzyme system degrading DNP of the cell nucleus is activated when oxidative phosphorylation of MCH is disturbed [7]. The fact that DNase I is activated in an aerated suspension of MCH may have an important bearing on the understanding of the mechanisms of the oxygen effect — potentiation of radiation disturbances in cells during an increase in the intensity of respiration of the irradiated animal.

We suggest that activation of mitochondrial phospholipase A during release of calcium from MCH can lead not only to activation of DNase I, but also to activation of DNase II localized in the lysosomes, through a disturbance of the integrity of the membrane of the organelles.

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KINETICS OF LIPID PEROXIDATION IN THE ISOLATED SURVIVING RAT LIVER

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The kinetics of accumulation of lipid peroxidation products (primary products, hydroperoxides; secondary products, malonic dialdehyde and "fluorescent pigments") in the isolated unperfused and previously perfused liver was investigated during aerobic incubation. During survival intensive accumulation of primary, secondary, and end products of lipid peroxidation was shown to take place, and its kinetics is extremal in character. The velocity of this process in the unperfused liver is much higher than in the previously perfused liver.

KEY WORDS: survival of the liver; lipid peroxides; hydroperoxides; diene conjugation; malonic dialdehyde.

One of the most important factors in the destruction of biological cell structures is free-radical oxidation of unsaturated fatty acids of the phospholipids of their biomembranes [1, 2]. In vivo, in normally functioning organisms, this process is always taking place but at a low level and under steady-state conditions. This is achieved through the presence of a complex multicomponent system in the tissues: biological antioxidants, enzymic protective systems, quenchers of singlet-excited oxygen, and systems regulating the supply of oxygen to membrane phospholipids [3, 8].

In isolated tissues during perfusion favorable conditions are created for lipid peroxidation (LPO) as a result of accumulation of H^+, a shift of the redox equilibrium toward reduction, disturbance of the bond between protein and lipid, accumulation of nonhemin iron, and a decrease in the content of biological antioxidants. This process may perhaps lie at the basis of the pathogenesis of ischemic lesions [2-5], for products of LPO can cause an increase in membrane permeability, oxidation of reduced thiols, inactivation of membrane-bound enzymes, polymerization of certain amino acids and proteins, and disintegration of cell metabolism [9, 13]. A detailed investigation of LPO in isolated tissues during survival could provide information on the mechanism of irreversible tissue damage after death and for the study of optimal methods of conservation of organs and tissues.

The object of this investigation was to study the kinetics of accumulation of primary molecular products (hydroperoxides and diene conjugates) and of secondary products of LPO (malonic dialdehyde, MDA) and of intermolecular cross-linkages between them and aminophosphatides ("fluorescent pigments") in the isolated unperfused and perfused rat liver during survival at 37°C for 24 h.

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