ROLE OF CHANGES IN LYSOSOMAL PERMEABILITY IN THE MECHANISM OF LOW TEMPERATURE INHIBITION OF PROTEIN SYNTHESIS IN THE RAT LIVER

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The writers showed previously that freezing and thawing tissues and cell populations [2, 3] leads to a sharp fall in the level of protein synthesis. Subsequent experiments on cell-free systems suggested that the primary role in the mechanism of low-temperature disturbance of the protein-synthesizing system of cells is played by secondary factors rather than direct injury to components concerned with translation: ribosomes, RNA, enzymes, etc. [4]. The most important of these secondary factors is evidently disturbance of the integrity of the lysosomes accompanied by liberation of hydrolytic enzymes into the cytoplasm [5].

To test this hypothesis the investigation described below was undertaken to study the action of rat liver lysosomes subjected to fast or slow freezing and thawing on the protein-synthesizing activity of a homologous cell-free system.

EXPERIMENTAL METHOD

Lysosomes were isolated from the liver of albino rats by differential centrifugation in 0.25 M sucrose, made up in 0.005 M Tris-HCl buffer, pH 7.4 [9]. After removal of the sucrose solution the lysosomes were resuspended in 0.15 M KCl solution and portions of the suspension, 4 ml in volume, were placed in polyethylene containers and frozen slowly in nitrogen vapor at the rate of 1-2°C/min to −196°C, or quickly – by immersion in liquid nitrogen. The rate of freezing in this case was 200-400°C/min. Rapid rewarming was carried out on a water bath at 37°C, slow rewarming at 20°C. To obtain supernatant (lysate) the samples were centrifuged at 30,000 g for 30 min. Total destruction of the lysosomes was achieved by treatment in 0.2% Triton X-100 [6]. Unsedimented RNase activity was determined by Shapot's method [7]. Protein was determined by Lowry's method [10].

Protein-synthesizing activity of the postmitochondrial supernatant from rat liver (S 15), which was used as the cell-free system for protein synthesis, was determined as described in [4], except that the S 15 was not passed through a column with Sephadex G-25 before addition to the cell-free system. This modification avoided the necessity of adding unlabeled amino acids to the cell-free system.

Besides S 15, the cell-free system contained the following components: ATP 1 mM, GTP 0.1 mM, creatine phosphate 10 mM, creatine-phosphate kinase 16 μg/ml, Tris-HCl, pH 5, 30 mM, KCl 120 mM, $MgCl_2$ 5 mM. A labeled amino acid, ^{14}C-leucine (240 mCi/mmole, Czechoslovakia) was used, and was added at the rate of 0.1 μCi per sample. The cell-free system was incubated at 37°C for 5 min. At the end of the reaction the samples were treated as described in [5] and radioactivity counted in toluene scintillator, by means of the SL-40 scintillation counter (France).

EXPERIMENTAL RESULTS

The experiments showed that lysed preparations of lysosomes subjected to slow freezing or thawing had the strongest effect on the protein-synthesizing activity of S 15 (Fig. 1A). Inhibition of incorporation of ^{14}C-leucine during fast freezing and slow thawing and during slow freezing and slow thawing amounted to 35.1 and

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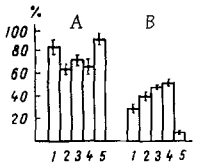

Fig. 1

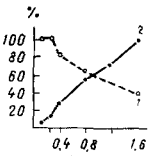

Fig. 2

Fig. 1. Effect of lysosomal lysates after freezing and thawing under different conditions on protein-synthesizing activity of cell-free extracts and on hydrolysis of RNA. A) Incorporation of ^{14}C-leucine into acid-insoluble product. Incorporation in control without addition of lysosomal lysate taken as 100%; B) hydrolysis of RNA. Hydrolysis of RNA in sample containing supernatant of Triton lysosomal lysate taken as 100%. 1) Lysosomal lysates subjected to fast freezing and fast thawing added to incubation medium; 2) fast freezing and slow thawing; 3) slow freezing and fast thawing; 4) slow freezing and slow thawing; 5) control.

Fig. 2. Protein-synthesizing activity of cell-free extract and hydrolysis of RNA in presence of Triton lysosomal lysate. 1) Incorporation of ^{14}C-leucine into acid-insoluble product. Incorporation in control taken as 100%; 2) hydrolysis of RNA. Hydrolysis of RNA in sample containing undiluted Triton lysosomal lysate taken as 100%. Abscissa, protein concentration of lysosomal lysate (in mg/ml).

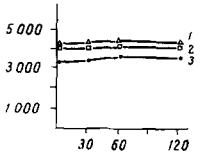

Fig. 3. Effect of lysosomal lysate subjected to low-temperature treatment and subsequent hypothermic storage on protein-synthesizing activity of cell-free extract. 1) Lysates of lysosomes subjected to fast freezing and fast thawing added to incubation medium; 2) to slow freezing and fast thawing; 3) control. Abscissa, duration of hypothermic storage (in min); ordinate, radioactivity (in cpm).

32.9% respectively. Slow freezing followed by fast thawing and, in particular, fast freezing and fast thawing had milder effects. The relationship thus observed correlates with the liberation of hydrolytic enzymes and, in particular, of RNases during freezing and thawing under the conditions investigated (Fig. 1B). However, when the data given in Fig. 1A, B are compared it will be noted that the RNase activity of the lysed lysosomes, whatever conditions of freezing and thawing were used, was greater than its inhibitory action on protein-synthesizing activity of the cell-free extract. This fact is confirmed also by comparing the effect of the Triton

lysate of lysosomes on incorporation of ^{14}C-leucine in a cell-free system and on RNA hydrolysis (Fig. 1). It may be that the difference observed is due to the presence in S 15 of inhibitors of lysosomal hydrolases, e.g., an inhibitor of RNase [8], and also to structural protection of RNA and other membrane-bound translation components in the composition of the polysomes [1].

Besides gross rupture of membranes, exposure to low temperatures can also cause latent injuries which develop in the course of time and lead to an increase in their permeability [11]. Since from the practical point of view conditions with rapid thawing are most frequently used, it was interesting to study the effects of lysed preparations of lysosomes subjected to fast or slow freezing, followed by fast thawing and then kept at 2-4°C. As will be clear from Fig. 3, the level of their injurious action for the whole period of time investigated remained practically unchanged.

The results of these experiments thus confirm the role of disturbance of lysosomal permeability in the inhibition of protein synthesis after exposure to low temperatures. The injurious action of the lysosomes is associated with disturbance of their permeability for hydrolytic enzymes during freezing and thawing, and it is evidently partly compensated in the tissues by the presence of hydrolase inhibitors and by structural protection of information macromolecules.

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