RAPID SPECTROPHOTOMETRIC DETERMINATION OF THERMO-RESISTANCE OF ANIMAL TISSUE LYSOSOMES

S. P. Syatkin and V. A. Frolov

UDC 612.014.2:576.311.344/.014.43

KEY WORDS: lysosomes, thermoresistance, rat liver.

The role of lysosomes in the pathogenesis of the overwhelming majority of diseases of animals and man has been conclusively proved [3]. Realization of the pathogenetic effect at the lysosomal level is associated with damage to the membranes of these particles and the release of their hydrolytic enzymes into the cytoplasm. Accordingly, the quantitative assessment of the degree of labilization of lysosomal membranes is important, but it is difficult because of the absence of any direct method of measuring the structural and functional state of lysosomes. Such an evaluation is done indirectly by determining the balance between different forms of acid hydrolases which are markers for this organelle [2]. The relative percentage of the nonsedimented form of enzyme activity relative to the total is regarded as a measure of integrity of lysosomes. The temperature of the incubation medium, in particular, is used as the harmful factor to be tested. Disadvantages of the method include the long duration and laboriousness of the analysis, the use of expensive reagents in short supply, and the low degree of specificity of determination because of differences in the degree of thermosensitivity of the enzymes themselves, which makes it difficult to compare results obtained with different acid hydrolases.

The aim of the present investigation was to develop a method of assessing thermoresistance of lysosomes isolated from animal tissues which will be free from the disadvantages mentioned above.

EXPERIMENTAL METHOD

Intact noninbred albino rats weighing 120-180 g, deprived of food for 48 h, were used. The animals were decapitated, the liver was removed and washed in ice-cold physiological saline, moisture was removed with filter paper, all connective-tissue was carefully removed, and the material was weighed. A homogenate was prepared in the ratio of 1:10 (weight of tissue to volume of homogenization medium). Homogenization was carried out in a Potter-Elvehjem glass homogenizer with Teflon pestle (gap 0.21 mm) for 90 sec at 1200 rpm in 0.33 M sucrose solution, pH 7.4, containing 1 mM EDTA. The lysosome-rich fraction was obtained by differential centrifugation [8] and resuspended in 0.7 M sucrose solution, pH 7.0, in the ratio of 1:0.2 (wet weight of tissue to volume) up to a concentration of lysosomal protein of 5 mg/ml, and it was stored at 0-4°C and used as the initial suspension.

Structural integrity of the lysosomes was judged from absorbance of the lysosomal suspension [5]. The initial suspension was added to a solution of 0.7 M sucrose, pH 7.0, with 1 mM EDTA, previously heated to the assigned temperature, in the ratio of 1:100-150 (v/v), so that the initial extinction value was about 0.4. This value was taken as 100% absorbance. The degree of destruction of the lysosomes was expressed as a percentage of this initial value. Changes in extinction were recorded continuously during 5 min of incubation in a constant-temperature cuvette (1 cm) at 520 nm on a Beckman (USA) recording spectrophotometer under programed control. The control sample contained the same components except lysosomes. The rate of destruction of the lysosomes was measured as the rate of decrease of optical density at 520 nm (ΔA_{520}/min) under programed control, during incubation of the samples under the same conditions but for 1 min. Values of incubation medium temperature were assigned over the range from 10 to 50°C.

Department of Pathological Physiology, Medical Faculty, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR T. T. Berezov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 9 , pp. 379-381, September, 1986. Original article submitted November 15, 1985.

 0007-4888/86/0009-1314$12.50

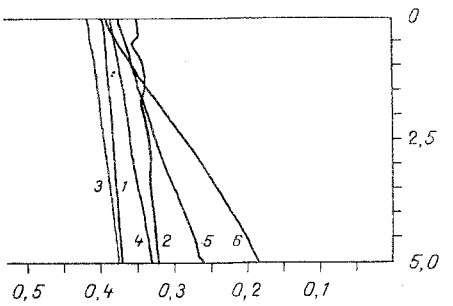

Fig. 1. Initial serial trace of change in A_{520} of suspension of intact lysosomes depending on incubation time and incubation medium temperature. Abscissa, optical density (A_{520}) of suspension of lysosomes measured at 520 nm (in relative units); ordinate, incubation time (in min). 1-6) Samples incubated at 10.3, 11.4, 20, 30, 37, 45, and 50°C respectively.

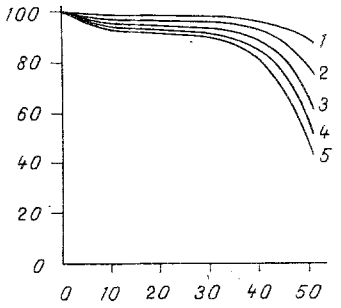

Fig. 2

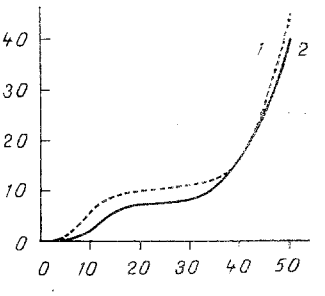

Fig. 3

Fig. 2. Dependence of optical density of suspension of intact lysosomes on incubation medium temperature at different times of incubation. Abscissa, incubation medium temperature (in °C); ordinate, percentage of initial value of A_{520}. 1-5) Incubation time of samples 1, 2, 3, 4, and 5 min respectively.

Fig. 3. Dependence of rate of destruction of lysosomes on incubation medium temperature and times of keeping initial suspension at 0-4°C. Abscissa, incubation medium temperature (in °C); ordinate, rate of fall of A_{520} expressed in ΔA_{520}/min. Samples kept at 0-4°C for 16 h (1) and 40 h (2).

EXPERIMENTAL RESULTS

An initial trace of changes in optical density of the lysosomal suspension during incubation under seven temperature programs for 5 min is shown graphically in Fig. 1. Since initial values of A_{520} for the graphs obtained do not coincide, for convenience of analysis changes in A_{520} with time were expressed as percentages of their initial value. It will be clear from Fig. 1 that the process of destruction of lysosomes under these conditions was linear in character, at least for 5 min. This is evidence that, within this range, it is acceptable to use any value of incubation medium temperature to characterize thermoresistance of lysosomes. Dependence of the degree of destruction of intact lysosomes on incubation medium temperature is multiphase in character (Fig. 2). For instance, between 0 and 10°C the degree of lysis of lysosomes is relatively small (in 5 min 7% are destroyed). This process later stabilizes and, despite an increase of temperature from 10 to 30°C, the increase in the degree of destruction was only 3%. The break of the curve occurred at a

temperature of 37°C. A further increase of temperature causes a sharp increase in the degree of destruction of the lysosomes and the relationship becomes parabolic in form. This phasic nature of the relationship was exhibited even more clearly still by dependence of the rate of destruction of lysosomes (kept at 0-4°C) on incubation medium temperature (Fig. 3).

The relationships characterizing thermoresistance of isolated intact lysosomes, which we obtained, are thus relatively simple and sufficiently informative. For instance, it follows from Fig. 1 that the lysosome suspension need only be incubated for 5 min under the condtions specified at t > 37°C to obtain easily measurable changes in A_{520}. Compared with the enzymic method, this reduces the total time of analysis from 5-6 h to 30 min. Thermosensitivity of lysosomes can be estimated quantitatively as a percentage of the initial value of A_{520} (Fig. 2) or relative to the initial rate of destruction of the particles (Fig. 3). To compare the action of different physicochemical factors on lysosomes in vivo and in vitro, parameters such as melting temperature (Tm) and half-disintegration time of the lysosomes ($t_{\frac{1}{2}}$) also are very convenient. The value of Tm can be calculated from data in Fig. 2 as the temperature at which changes in A_{520} amount to 50% of the maximal value, whereas $t_{\frac{1}{2}}$ can be calculated from data in Fig. 1 as the time during which the number of lysosomes is reduced by half. The fact that lysis of lysosomes is a linear function of time shows that quantitative proportions of the components of the incubation medium were correctly chosen. The presence of a steady-state region on the melting curves (Figs. 2 and 3) between temperatures of 10 and 30°C is in good agreement with the view that "melting" of lipids does not take place at a strictly definite temperature, but over a certain temperature range. Characteristically the most significant changes in structure of lysosomes take place after 5 min in incubation medium with temperatures over 37°C. This is in good agreement with the positive therapeutic effect of hyperthermia on tumor tissue and it may be one of the mechanisms of this action of hyperthermia. In these experiments local heating to 42°C and short-term exposure for between 15 and 60 min were used. The most significant changes in stability of the lysosomal membranes were observed after 15 min [4]. Longer exposure, as results obtained by some workers have shown, was ineffective [6], but according to results obtained by others, changes were not significant [4]. This contradiction is evidently due to technical errors connected with inactivation by heat of the enzymes used as the research tool. The suggested method is free from this disadvantage. The results also help to explain the positive action of antipyretic drugs on the grounds that a general lowering of body temperature stabilizes the lysosomes and thereby prevents any manifestation of their harmful action. Another possibility is that, by stabilizing lysosomal membranes under conditions of hyperthermia, antiinflammatory drugs prevent the development of the inflammatory process [1, 7].

Compared with enzymic methods, this method significantly shortens the time of analysis and reduces its laboriousness, it economizes in expensive reagents, and increases the specificity and universality of determination. Thermoresistance of isolated lysosomes can be used as an indicator of the structural and functional state and of the degree of the harmful action of various physicochemical factors on them in vivo and in vitro. Expression of the change in A_{520} and ΔA_{520}/min as a percentage, or the melting temperature (Tm) and half-disintegration time ($t_{\frac{1}{2}}$) of the lysosomes can be used as meaure of this action.

LITERATURE CITED

1. N. A. Mokhort, E. D. Molyuk, and T. L. Sakun, Farmakol. Toksikol., No. 4, 64 (1979).
2. A. A. Pokrovskii, and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
3. S. S. Debov (ed.), Structures and Functions of Lysosmes [in Russian], Novosibirsk (1980).
4. S. E. Ul'yanenko and B. P. Surinov, Byull. Éksp. Biol. Med., No. 7, 39 (1984).
5. P. Badenoch-Jones and H. Baum, Biochem. J., 142, 1 (1974).
6. W. C. Dewey, G. L. Freeman, G. P. Raaphors, et al., in: Cell Biology of Hyperthermia and Radiation, New York (1980), p. 580.
7. J. R. Joffe, Brit. J. Pharmacol., 72, 603 (1981).
8. R. Stahn, K. P. Maier, and K. Hannig, J. Cell Biol., 46, 576 (1970).