Influence of p_H on the Autolysis of Intracellular Proteins in Homogenates of Rat Spleen and Liver

A. SUHAR and M. FURLAN

Nuclear Institute "Jožef Stefan", Ljubljana, Yugoslavia

(Z. Naturforsch. 22 b, 324—326 [1967]; eingegangen am 28. September 1966)

The breakdown of endogenous proteins in the homogenates of rat liver and spleen, was investigated. The shape of the p_H -activity curves is shown to depend on the time of incubation. A pronounced effect of age of animals was observed with rat spleen homogenates. In mixed homogenates of liver and spleen, the production of hydrolytic products is slower than in pure spleen homogenates.

Intracellular acid proteinases — cathepsins are known to play an important role in the post-mortem autolysis and necrosis of animal tissues¹. Increased proteolytic activities were found in various physiological and pathological conditions, i. e. in growing and regressing tumours^{2, 3} in rat mammary gland during pregnancy, lactation and involution⁴, in regenerating and regressing *Xenopus* tail^{5, 6} in dystrophic muscle^{7, 8}, as well as in various organs of experimental animals treated with growth hormone⁹, vitamin A¹⁰, prednisolone¹¹, hydrocortisone¹², and after whole-body irradiation¹³. Increased activity of intracellular proteinases is certainly related to the enhanced breakdown of endogenous proteins; very little is, however, known about the mechanism and kinetics of the intracellular protein digestion. Purified cathepsins exert different p_H optima towards different protein substrates^{14, 15}. Moreover, several p_H optima were observed in individual tissues by the use of a single substrate^{12, 15, 16}. It has been suggested that the cells contain protein-specific proteinases¹⁷; therefore, the assay of proteolytic activity with hemoglobin can not give a complete picture of the intracellular protein catabolism.

The experiments described in this report were designed to determine the influence of p_H and time on the in vitro autolysis of intracellular proteins in the homogenates of rat spleen and liver.

Materials and methods

Male Wistar rats were used. The tissues were excised, blotted, weighed and homogenized in a Potter-Elvehjem homogenizer with a teflon pestle. Livers and spleens were homogenized in 9 and 19 volumes of 0.25 M sucrose solution containing 0.1% Triton X-100, respectively. Aliquots of the obtained homogenates were mixed with sodium acetate buffer solutions of p_H 2 to 7, and incubated for 10, 60, and 180 minutes at 37 °C. The reaction was stopped, and the undigested proteins were precipitated by the addition of 5% trichloroacetic acid. The concentration of digestion products in the filtrate was assayed according to the method of ANSON¹⁸. The results are expressed in extinction units of the blue colour obtained with the Folin-Ciocalteu phenol reagent. Nitrogen was determined by a modified KJELDAHL method¹⁹.

Results and discussion

Changes in hydrolysis rate of rat liver homogenate with p_H are shown in Fig. 1. It is evident that the

¹ H. C. BRADLEY, *Physiol. Rev.* **18**, 173 [1938].

² G. BENZ, *Oncologia* **12**, 128 [1959].

³ B. SYLVÉN and H. MALMGREN, *Acta radiologica* [Stockholm] Suppl. **154**, 1 [1957].

⁴ A. L. GREENBAUM and F. C. GREENWOOD, *Biochem. J.* **56**, 625 [1954].

⁵ E. M. DEUCHAR, R. WEBER, and F. E. LEHMANN, *Helv. physiol. pharmacol. Acta* **15**, 212 [1957].

⁶ R. WEBER, *Rev. Suisse Zool.* **64**, 326 [1957].

⁷ R. J. PENNINGTON, *Biochem. J.* **88**, 64 [1963].

⁸ A. L. TAPPEL, H. ZALKIN, K. A. CALDWELL, I. D. DESAI, and S. SHIBKO, *Arch. Biochem. Biophysics* **96**, 340 [1962].

⁹ J. C. MATHIES, L. PALM, and O. H. GAEBLER, *Endocrinology* **49**, 571 [1951].

¹⁰ J. T. DINGLE, I. M. SHARMAN, and T. MOORE, *Biochem. J.* **98**, 476 [1966].

¹¹ W. NAGEL and F. WILLIG, *Z. Hoppe-Seyler's physiol. Chem.* **324**, 45 [1961].

¹² M. FURLAN, *Strahlentherapie*, in press.

¹³ D. LEBEZ, M. FURLAN, M. ANTONIJEVIĆ, and M. KOPITAR, IInd Int. Congress of Radiat. Res., Harrogate 1962.

¹⁴ E. M. PRESS, R. R. PORTER, and J. CEBRA, *Biochem. J.* **74**, 501 [1960].

¹⁵ V. TURK, Ph. D. THESIS, Univ. Ljubljana 1965.

¹⁶ A. L. DOUNCE and R. UMANA, *Biochemistry* **1**, 811 [1962].

¹⁷ M. FURLAN, *Enzymologia* **31**, 9 [1966].

¹⁸ M. L. ANSON, *J. gen. Physiol.* **20**, 565 [1937].

¹⁹ C. DUMAZERT and Y. MARCELET, *Bull. Soc. Chim. biol.* **20**, 201 [1938].

Fig. 1. Effect of pH on the hydrolysis of endogenous proteins in homogenates of rat liver (6- to 12-month-old animals): mean value of 5 experiments $\pm$ S.E.

shape of the pH -activity curve depends on the time of incubation. After 10-minute incubation, a distinct pH optimum was observed at pH 2.5. This peak was shifted to higher pH values after 1-hour and 3-hour incubation. Comparison of the relative heights of the three pH -activity curves indicates that the rate of protein digestion falls with time of incubation. This fall could be attributed either to an inactivation of active cathepsins, to the inhibition by the reaction products, or to the changes in the concentration and structure of endogenous protein substrates during the course of hydrolysis. No changes in the pH -activity curves of rat liver homogenates were found with age, when 6-month-old rats were compared to 12-month-old animals. A pronounced effect of age on the pH -activity curves was, however, observed with rat spleen homogenates (Figs. 2 and 3). Influence of age on the activity of intracellular enzymes has been reported^{20, 21}. Our results show two optima at pH 2.5 and 4.0 in 6-month-old rats, whereas only one activity peak at pH 4.0 was found in 12-month-old animals. Effect of incubation time on the pH -activity curves of spleen homogenates was similar to that observed with liver homogenates: the optima were shifted to higher pH values and the extinction increase was not proportional to the time of incubation.

Our results show that the pH optima for the digestion of endogenous proteins differ from the values reported for hemoglobin hydrolysis^{14, 18, 22, 23}. The peak at pH 2.5 could be compared to the pH optimum of cathepsin E towards serum albumin sub-

Fig. 2. Effect of pH on the hydrolysis of endogenous proteins in homogenates of rat spleen (6-month-old animals): mean value of 5 experiments $\pm$ S.E.

Fig. 3. Effect of pH on the hydrolysis of endogenous proteins in homogenates of rat spleen (12-month-old animals): mean value of 5 experiments $\pm$ S.E.

strate²⁴. Proteinase activity maximum of cathepsin D was found at pH 4.2 with serum albumin as substrate¹⁴.

In further experiments, the autolysis of endogenous proteins in mixed homogenates of rat liver and spleen, was investigated. Aliquots of a homogenate containing 10% w/v of liver tissue and 5% w/v of spleen tissue (6-month-old rats) were incubated at different pH 's as described for pure liver and spleen homogenates. The extinctions were com-

²⁰ D. LEBEZ, V. TURK, and M. FURLAN, to be published.

²¹ C. H. BARROWS, JR., *Federat. Proc.* **15**, 954 [1956].

²² A. SCHÄFFNER and M. TRUELLE, *Biochem. Z.* **315**, 391 [1943].

²³ M. FURLAN, Ph. D. THESIS, Univ. Ljubljana 1964.

²⁴ C. LAPRESLE and T. WEBB, *Biochem. J.* **84**, 455 [1962].

pared to the values obtained by summing up the results of separate incubations of both liver and spleen homogenates (Figs. 4 and 5). It is evident that the shape of p_H -activity curve is similar but the hydrolysis rate in mixed homogenate is much lower than the theoretical values. The extinctions of the mixed homogenate are even less than those found with pure spleen homogenate (Fig. 2). It can be concluded that the liver contains inhibitors of spleen intracellular proteinases.

Our results confirm that the mechanism of protein catabolism in the cells is extremely complex; the digestion of intracellular proteins depends upon the concentration of active proteinases and suscep-

Fig. 4. Effect of p_H on the hydrolysis of endogenous proteins in mixed homogenates of rat liver and spleen (6-month-old animals).

Fig. 5. Sum of the hydrolytic rates for the breakdown of liver and spleen endogenous proteins; the results were obtained by summing up the values from Figs. 1 and 2 (6-month-old rats).

tible protein substrates, as well as on the interactions among them, which are influenced by endogenous inhibitors.

This work was supported by the Yugoslav Nuclear Energy Commission, Contract No. 03-803/5. We wish to thank Mrs. V. ŠTRUKELJ for valuable technical assistance.