PRO EXPERIMENTIS

Mechanism of Elastolysis by Pancreatic Elastase

Acetate extracts of pancreas powder contain an enzyme system which dissolves elastin from aorta media, tendon and also heat-contracted collagen[1]. In order to study the mechanism of elastolytic activity, we worked out a simple method based on the spectrophotometric measurement of the rate of dissolution of 'azoelastin', prepared by the method used by OAKLEY *et al.*[2] for the preparation of azocollagen. This comprises coupling elastin (from aorta media, BANGA[3]) with diazobenzidine-naphtoldisulphonic acid (details of the method will be given in another paper).

'Azoelastin' as well as elastin is only very slowly digested by crystalline trypsine[4]: at pH 9·5–9·7 (glycine-NaOH-buffer, 0·1 *M*)[5] 2 mg azoelastin is dissolved in 24 h by 0·88 mg trypsine. Crystalline chymotrypsine[4] works somewhat better: 1·2 mg of this enzyme dissolved 17 mg azoelastin in 2 h and 74 mg in 7 h at 35°C, in the same conditions as for trypsine. The mixture of these two enzymes dissolved the same quantity. Crystalline pepsine[4] dissolved azoelastin completely in a few hours at pH 1–2. The proteolytic factor (now called elastase by BANGA[6]) of one of our elastase preparations (obtained by the Banga-Balo method[3]) had a proteolytic activity equivalent to 229 γ of crystalline trypsin per milligram total protein, as determined by NORTHROP's hemoglobine-method[7]. This activity is somewhat lower than that found by PARTRIDGE and DAVIS[8].

The same preparation dissolved 18·6 mg azoelastin per milligram enzyme protein in 30 min at 35°C and about 10 mg of this enzyme gave complete dissolution of 200 mg azoelastin in 2 h.

The elastin-elastase system is suitable for the study of the heterogenous enzyme kinetics. The first step of the enzymatic dissolution process seems to be the rapid adsorption of enzyme onto the insoluble substrate. This is demonstrated by the following experiment: different quantities of elastase are added to the same quantity (200 mg) of azoelastin in 3 ml acetate buffer 0·1 *M* pH 4·9 with water added to 5 ml. After 30 min shaking at 20°C the azoelastin is centrifuged down and washed out twice with 3 ml acetate buffer, siphoned and kept overnight at 0°C. After heating to 35°C 4 ml glycine-NaOH buffer pH 9·7, 0·1 *M* is added with 2 ml water and elastolysis is followed spectrophotometrically at 520 mμ. The first figure gives some typical curves of this experiment together with control curves. It is clear that preincubation with the adsorbed enzyme resulted in a modification of the substrate, the lag period of the digestion disappears. The second figure gives the slopes of the linear portions of the curves as a function of total initial enzyme concentration, for the adsorption experiment and the control series. At low enzyme concentrations dissolution is faster in the control series, but for higher

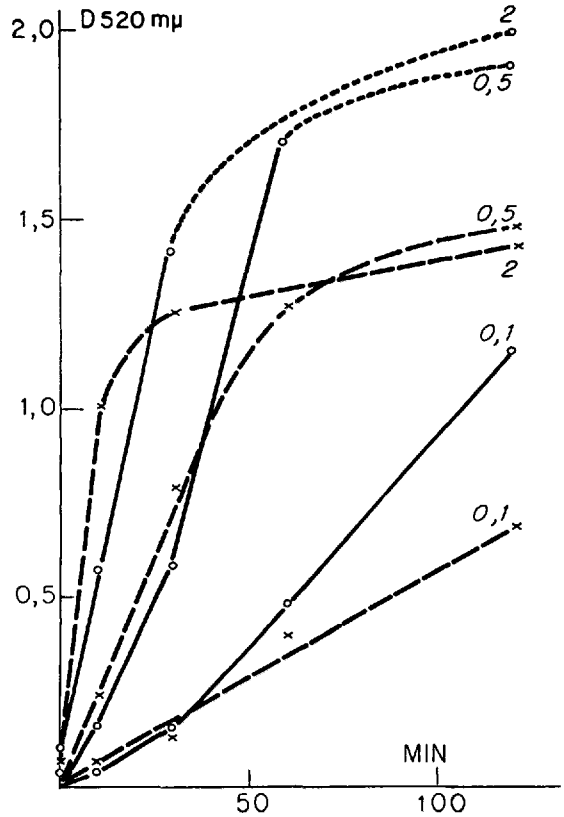

Fig. 1. – Kinetics of dissolution of azoelastin by preadsorbed enzyme (- - -) and freshly added enzyme (——). Ordinates: optical density at 520 mμ. The figures on the curves indicate the quantity of enzyme in ml added to 200 mg azoelastin. In the adsorption experiments non adsorbed enzyme was removed.

enzyme concentrations adsorbed enzyme acts much more rapidly. This could be interpreted as a preliminary

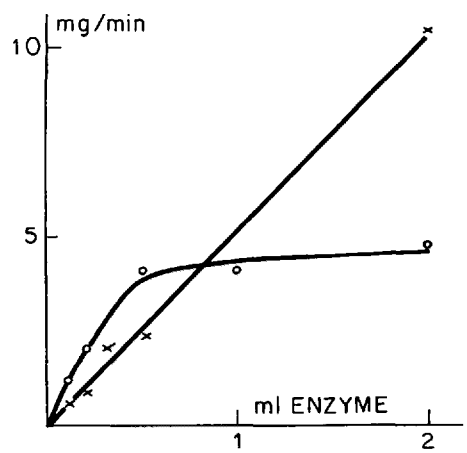

Fig. 2. – Abscissa: millilitres of enzyme added to 200 mg azoelastin. In adsorption-experiments the non-adsorbed enzyme was removed. Ordinate: speed of elastolysis in mg azoelastin dissolved per minute at 35°C, pH 9·6, o——o freshly added enzyme, x——x preadsorbed enzyme.

modification of azoelastin during the incubation by a slow enzymatic process of low activation energy rendering

[1] J. BALO and I. BANGA, Biochem. J. *46*, 384 (1950). – I. BANGA, Nature *172*, 1099 (1953).

[2] C. L. OAKLEY, G. HARRIET, and W. E. VON HEYNINGEN, J. Path. Bact. *58*, 229 (1946).

[3] I. BANGA, Acta physiol. Acad. Sci. Hung. *3*, 317 (1952).

[4] We thank Worthington Biochemicals, New Jersey, for the generous gift of crystalline trypsin, chymotrypsin and pepsin.

[5] J. BALO and I. BANGA, Biochem. J. *46*, 384 (1950).

[6] I. BANGA and J. BALO, Nature *178*, 310 (1956).

[7] J. H. NORTHROP, M. KUNITZ, and R. M. HERRIOTT, *Crystalline Enzymes* (Columbia Univ. Press 1948), p. 307.

[8] S. M. PARTRIDGE and H. F. DAVIS, Biochem. J. *61*, 21 (1955).

accessible the substrate to the proteolytic component. It is not impossible that this preliminary reaction, taking place during the lag phase, could be the mucolytic process mentioned by BANGA and BALO[6]. We could verify that the adsorption mechanism holds for the dissolution of non-diazotised aorta-elastin too.

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Résumé

Nous présentons une étude du mécanisme de l'élastolyse par une élastase partiellement purifiée du pancréas. La méthode utilisée consiste dans le dosage spectrophotométrique de l'«azoelastine» dissoute. Cette protéine n'est que très lentement hydrolysée par la trypsine et chymotrypsine cristallisées. L'élastase possède une activité protéasique considérable. L'enzyme est très rapidement adsorbée sur l'élastine. Après une incubation à pH 4,5 avec l'enzyme adsorbée, le temps de latence de l'élastolyse disparaît et la rapidité de la réaction devient proportionnelle à la concentration en élastase.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

The Metabolism of the Proteins of the Brain*

By A. LAJTHA, S. FURST**, and H. WAELSCH

Of the major components of the brain, least attention has been paid to the protein constituents. The large concentration of lipids and the process of myelination, unique for the nervous tissue and so easily visualized by histological techniques, have for many years attracted the interest of biochemists. With the development of our understanding of the role of carbohydrates in the energy metabolism of living tissue, the last 25 years witnessed an ever increasing concern with this aspect of intermediary metabolism of the brain. Only recently have the amino acids and proteins of the central nervous system been subjected to more intensive study, but these investigations have been and are essentially analytical in nature. Hardly any other organ system offers as many fascinating and far reaching implications for the function of its component proteins as does the nervous system.

This laboratory has for many years been interested in the metabolism of the amino acids and proteins of the nervous system, and we should like to summarize in this report our studies dealing with the turnover of the proteins of the whole brain, of different parts of the brain, and of different cell fractions[1]. Incidental to these investigations, studies of the penetration of lysine into the brain of young and adult animals as well as observations on protein metabolism in the immature brain have been made.

The study of the turnover of brain proteins by the determination of the rate of uptake of an isotopically labelled amino acid is made difficult by the existence of the blood-brain barrier which decreases the rate of transfer of many metabolites from the circulating blood to the brain. Some compounds are, for all practical purposes, excluded from the brain[2]. For example, no increase of the glutamic acid concentration in brain could be detected after a 30 fold increase of this amino acid in the blood. On the other hand, a significant, although small, increase of the glutamine concentration in brain was found after intravenous administration of the amide to rats or mice[3]. It is not surprising that isolated observations of lack of incorporation of amino acids, parenterally administered, led to the conclusion that the brain proteins have a very slow turnover[4]. When, on the other hand, amino acids were introduced directly into the subarachnoidal spaces, in order to avoid the blood-brain barrier, considerable incorporation into proteins was observed[5].

The design of the experiments.—For reasons to be discussed below, it appeared desirable to select the physiological path of supply of amino acids to the brain, i.e., from the circulating blood. Lysine was chosen as the test amino acid, there being reason to believe that this basic amino acid would penetrate the blood-brain barrier at a faster rate than an acidic or neutral one[6]. The technique followed, with minor variations, in the experiments to be reported, was as follows:

Lysine C^{14} was injected intravenously or intraperitoneally into mice and monkeys, and the brains, livers and other organs were removed after definite time intervals. The protein fractions obtained by precipitation with trichloroacetic acid and extraction with organic

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[2] H. WAELSCH in *Biochemistry of the Developing Nervous System* (H. WAELSCH ed., Academic Press, Inc., New York 1955).

[3] P. SCHWERIN, S. P. BESSMAN, and H. WAELSCH, J. biol. Chem. *184*, 37 (1950).

[4] H. BORSOOK and C. L. DEASY, Ann. Rev. Biochem. *20*, 209 (1951).

[5] F. FRIEDBERG, H. TARVER, and D. GREENBERG, J. biol. Chem. *173*, 355 (1948). – M. K. GAITONDE and D. RICHTER, Biochem. J. *59*, 690 (1955).

[6] H. WAELSCH in *Biochemistry of the Developing Nervous System* (H. WAELSCH ed., Academic Press, Inc., New York 1955). – P. SCHWERIN, S. P. BESSMAN, and H. WAELSCH, J. biol. Chem. *184*, 37 (1950).