Cathepsin A in Human Brain and Spleen

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A striking loss of cathepsin A activity occurs when relatively crude preparations of the enzyme are maintained at even a mildly basic pH value. This may explain the failure of others to detect the enzyme.

Cathepsin A (EC 3.4.2.-) catalyses the release of tyrosine from Z*-Glu-Tyr at acid pH and does not require thiols for activity (Fruton & Bergmann, 1939; Lichenstein & Fruton, 1960). Although this lysosomal (Shibko & Tappel, 1965; Coffey & de Duve, 1968) hydrolase was one of the first components of the cathepsin complex of bovine spleen to be described, the status of the enzyme as a tissue proteinase has been questioned (Iodice, 1967; Barrett & Dingle, 1971). However, more recent evidence (Logunov & Orekhovich, 1972) appears to substantiate the earlier data (Lichenstein & Fruton, 1960), and it seems likely that cathepsin A displays at least limited endopeptidase activity. Despite the earlier reports, Press *et al.* (1960) and Marks & Lajtha (1965) have been unable to detect the enzyme with any certainty in either spleen or brain respectively. In the present communication we show that these negative findings are probably due to the conditions that were used to extract the enzyme. Water was used for extraction when cathepsin A was successfully separated (Fruton & Bergmann, 1939; Lichenstein & Fruton, 1960) from spleen, whereas only traces of activity (Press *et al.*, 1960) were obtained when a basic medium (0.1M-sodium acetate) was used for the overnight extraction. Although cathepsin A-like activity was detected in a qualitative survey of calf brain peptidases (Brecher, 1963), a more recent report (Marks & Lajtha, 1965) is quoted as evidence that the enzyme is absent from the central nervous system (Mycek, 1970). In this latter work (Marks & Lajtha, 1965) an acetone-dried powder of rat brain was extracted overnight at pH 7.6. We have therefore reinvestigated the relationship between the pH of the extraction media and the recovery of cathepsin A activity from both brain and spleen.

Methods

Human brain and spleen were obtained within 24 h of death from patients with no history of lymphatic or neurological disorders. The deep-frozen tissues

* Abbreviation: Z-, benzyloxycarbonyl-.

were thawed and homogenized in 2 vol. of ice-cold 10mM-sodium phosphate buffer containing 8mM-2-mercaptoethanol, adjusted to final pH 6.5. After sonication the homogenate was clarified by centrifugation at high speed, essentially as described by Bowen & Radin (1969). The supernatant or dispersible fraction obtained in this way from brain contained more than half the total cathepsin A activity and is referred to as crude cathepsin A. Samples of each preparation were adjusted to the required pH (Fig. 1) by adding 0.13 vol. of the appropriate 0.75M buffer (sodium acetate for pH 4.5-5.5, sodium phosphate for pH 6-7.6, and sodium phosphate, pH 8, plus NaOH for pH 8-9). The mixtures were diluted with water to give a final concentration equivalent to 200mg original wet wt. of tissue/ml and were then maintained as described in Fig. 1. Before assay of the enzyme activity the pH was adjusted to pH 5.5 by adding dilute HCl. The extracts were further diluted with water to give respectively a final concentration equivalent to 35mg and 70mg of spleen and brain original wet wt./ml. Cathepsin A activity was assayed by a modification of the method of Iodice *et al.* (1966). The enzyme solution (0.2ml) with (final concentrations) 4mM-iodoacetic acid (sodium salt), 10mM-EDTA, 75mM-sodium acetate buffer, pH 5.5, and 13.3mM-Z-Glu-Tyr as substrate were included in the reaction mixtures (final vol. 0.5ml). In addition to controls without substrate a small correction was necessary for the hydrolysis of substrate in the absence of enzyme. Because of the low activity in brain (Fig. 1) preparations from this tissue were incubated for 16 h. The time-course of the reaction (established with unfractionated brain homogenates) remained linear for at least 16 h, and the observed activity was essentially directly proportional to tissue weight (from 4.5 to at least 15mg of brain original wet wt./tube).

Results and discussion

In the enzyme assay iodoacetate was added to inhibit selectively a thiol-requiring Z-Glu-Tyr hydrolase that was present in both spleen and brain.

Fig. 1. Effect of maintaining crude human cathepsin A at various pH values

Samples of cerebral grey matter (o) and spleen (●) were stored for 22 h at 7–8°C. The arrows indicate the pH values used by workers who failed to detect activity with any certainty (Press *et al.*, 1960; Marks & Lajtha, 1965). When assayed fresh the enzymes derived from 1 g of either grey matter or spleen catalysed the release of 0.64 and 24.2 μ mol of amino acid from Z-Glu-Tyr/h respectively. A similar ratio of activities is given by whole homogenates; grey matter contains 1–2 times as much activity as white matter.

Although this second enzyme is identical with catheptic carboxypeptidase A in its sensitivity to Zn^{2+} , iodoacetate (Greenbaum & Sherman, 1962) and heating (McDonald *et al.*, 1972), the pH optimum (pH 4.25) of relatively crude preparations is somewhat higher (D. M. Bowen & A. N. Davison, unpublished work). EDTA was added to inhibit metal ion-requiring peptidases and was without effect on purified cathepsin A from brain. The enzyme that we have isolated from brain (D. M. Bowen & A. N. Davison, unpublished work) is identical with mammalian cathepsin A with respect to optimum pH (5–5.5), heat-lability, a greater rate of hydrolysis of Z-Glu-Tyr than of Z-Glu-Phe and inhibition by 1 mM-di-isopropyl phosphorofluoridate but not by 1 mM-iodoacetate (Lichenstein & Fruton, 1960; Shibko & Tappel, 1965; Iodice *et al.*, 1966; Logunov & Orekhovich, 1972; McDonald *et al.*, 1972). However, the cathepsins from spleen and brain do not have identical chromatographic properties (D. M. Bowen & A. N. Davison, unpublished work). Other experiments demonstrate that the activity we have detected in brain is not due to contamination by blood or to bacterial growth during incubation. In addition, the cathepsin A activity that was obtained by using ad-

mixtures of either blood and brain or spleen and brain were additive, indicating that the low activity in brain is not due to an inhibitor.

The effect on activity of maintaining relatively crude cathepsin A at various pH values is shown in Fig. 1. The salient result is the striking loss of activity incurred by maintaining the enzyme from both cerebral grey matter and spleen at even a mildly basic pH value. In other experiments, with cerebral white matter and a slightly longer storage period, essentially all the activity was lost. Similarly when crude cathepsin A from brain is subjected to ion-exchange chromatography at pH 8, as described by Matsunaga *et al.* (1969), the recovery of activity is consistently very low, whereas the recovery is complete from columns developed at acidic pH values (D. M. Bowen & A. N. Davison, unpublished work). The loss of activity at mildly alkaline pH values appears to explain the failure of previous investigators (Press *et al.*, 1960; Marks & Lajtha, 1965) to detect the enzyme. Further, it suggests that the alkaline pH of the dialysis medium rather than dialysis as such was responsible for the loss of activity observed when bovine cathepsin A was dialysed at pH 8 (Iodice *et al.*, 1966). Our observations may also explain those of Matsunaga *et al.* (1969), who found that angiotensinase isolated after dialysis at mildly acidic pH values was very much richer in cathepsin A than was the enzyme prepared at pH 8, thus supporting the view (Yang *et al.*, 1968, 1970) that angiotensinase and cathepsin A are different enzymes. Although our results show that brain contains cathepsin A, the concentration in spleen is very much higher than that in cerebral white matter (Fig. 1). In inflammatory conditions of the brain measurement of cathepsin A activity may therefore serve as a useful index of cellular infiltration.

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