INHIBITION OF HUMAN LIVER CATHEPSIN L BY α-THIOL PROTEINASE INHIBITOR

M. PAGANO and R. ENGLER

Laboratoire de Radio-Immuno-Biochimie, UER Broussais-Hôtel-Dieu, 15, rue de l'Ecole de Médecine, 75270 Paris Cédex 06, France

Received 23 October 1981

1. Introduction

A protein inhibitor specific for thiol proteinases is present in human serum [1]. Two forms of this inhibitor have been purified [2,3] and named α-TPI. A thiol proteinase inhibitor has been demonstrated in human urine and this has been shown to be a degradation product of the plasma inhibitors [4]. The plasma protein strongly inhibits papain and ficin but inhibits cathepsin B, a typical lysosomal thiol proteinase, only weakly [1,3]. Here, we describe the inhibition of cathepsin L, another lysosomal thiol proteinase [5], by this plasma inhibitor. The results obtained suggest that one of the physiological functions of α-TPI is to inhibit cathepsin L.

2. Materials and methods

Ascites fluid or pleural fluid was used for the preparation of α-TPI. Human livers free from disease were used for the preparation of cathepsin L. These starting materials were stored at −20°C until required. Papain, ficin, α-casein, BANA, Arg-N-Nap, Leu-N-Nap, ammonium sulphamate, sodium nitrite, *N*-(1-naphthyl)-ethylenediamine, β-naphthylamine were purchased from Sigma (St Louis MO).

CNBr-activated Sepharose 4B and Sephadex G-75 (superfine) were supplied by Pharmacia Fine Chemicals (Uppsala). CM-cellulose (CM 32) and Ultrogel ACA 44 were purchased from Whatman (Maidstone Kent) and IBF (Gennevilliers), respectively. Aldolase activity was assayed with the Boehringer UV system (Mannheim).

The activity of papain was measured using BANA as substrate. For cathepsin L, the activity was tested using BANA, azocasein, aldolase, Arg-N-Nap and Leu-N-Nap as substrates. Azocasein was prepared by treating α-casein with diazotized sodium sulphanilate according to [6]. The inhibition of papain by α-TPI was assayed using BANA as substrate and for cathepsin L the inhibition by α-TPI was carried out with azocasein as substrate. The experimental conditions and the procedure for the activity measurements and the inhibition tests are described in [7]. Acrylamide-gel electrofocusing was performed according to [8] and SDS acrylamide-gel electrophoresis according to [9].

2.1. *Preparation of α-TPI*

α-TPI was purified as in [2]. The starting material was submitted to acid precipitation and, after centrifugation, the supernatant was neutralized. This was followed by affinity chromatography of the supernatant on a column of ficin–Sepharose (2 cm × 5 cm): 20 ml acid-treated fluid were used for each affinity chromatography. The 2 forms of α-TPI were fractionated on a column of Ultrogel ACA 44 (3 cm × 70 cm). During the different stages of the purification α-TPI was assayed for its inhibition capacity against papain. The concentration of the purified inhibitor was calculated from the absorbance at 280 nm by using $E^{1\%}_{1\,cm} = 5.9$ [3] and an M_r of 90 000 (α-TPI (1)) or 175 000 (α-TPI (2)) [2,3,10].

2.2. *Preparation of human liver cathepsin L*

The enzyme was purified by the method described for rat liver cathepsin B.

After homogenization of a piece of human liver

Abbreviations: TPI, α-thiol proteinase inhibitor; CL, cathepsin L (EC 3.3.22.15); BANA, *N*-benzoyl-D,L-arginine-2-naphthylamide–hydrochloride; Arg-N-Nap, L-arginine-2-naphthylamide–hydrochloride; Leu-N-Nap, L-leucine-2-naphthylamide–hydrochloride

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

(200–250 g) with Triton X-100 (0.2%, v/v) and acid precipitation at pH 3.8, the soluble material was extracted by stirring overnight at 4°C. The insoluble fraction was removed by centrifugation at 16 300 × *g* for 30 min. This was followed by precipitation with ammonium sulphate (2–3 M). After dialysis and centrifugation, the brown supernatant was applied to a CM 32 column equilibrated with 0.1 M sodium acetate buffer (pH 5.30). Cathepsin B emerged in the breakthrough peak. Cathepsin L, absorbed on the ion-exchange resin, was eluted with 0.15 M phosphate buffer (pH 7.50) according to [11]. Cathepsin L was isolated by a single gel filtration on Sephadex G-75 (superfine). For cathepsin B two gel filtrations under the same conditions were required. The protein concentration of purified cathepsin L was determined as in [12] with bovine serum albumin as standard. The absorption coefficient $E^{1\%}_{280\ \mathrm{nm},\ 1\ \mathrm{cm}}$ was calculated on this basis. The M_r of the enzyme was measured by polyacrylamide gels electrophoresis in the presence of 1% (w/v) SDS and 2% dithiotreitol.

3. Results

3.1. *Analysis of α-TPI and CL*

The purified inhibitor was studied by gel electrofocusing. α-TPI (1) exhibited a band at pI 4.85 when the pH gradient was made with ampholines ranging between pH 3.50–10. When the experiment was done at pH 4.0–6.5 two bands at pI 4.80 and 4.95 were seen (fig.1): the heterogeneity of α-TPI (1) has been reported by different authors [2,3,10]. α-TPI (2) showed one band at the bottom of the gel and a pI of 3.60 could be measured. Our results agree with [2] on the different molecular forms of α-TPI.

Purified cathepsin L was submitted to acrylamide gel electrofocusing. Over a gradient of pH 3.5–10 this enzyme exhibited 2 bands at pI 5.50 and 5.70 (fig.1). An M_r of 30 000 was found on SDS–polyacrylamide gels (fig.2). There is no report on the properties of human liver CL though the properties of the rat liver enzyme have been described [5]. The human enzyme had similar catalytic properties to those of the rat enzyme (table 1): a high proteolytic activity azocasein and a very low activity with BANA as substrate (a good substrate for the endopeptidase activity of cathepsin B and cathepsin H). Our preparation had no activity with Arg-N-Nap and Leu-N-Nap as substrates, used for the measurement of the aminopeptidase activity of cathepsin H [13,14]. On this basis, it may be taken that our purified cathepsin L is free from cathepsin B and cathepsin H activities. Aldolase inactivation has been reported as a specific property of

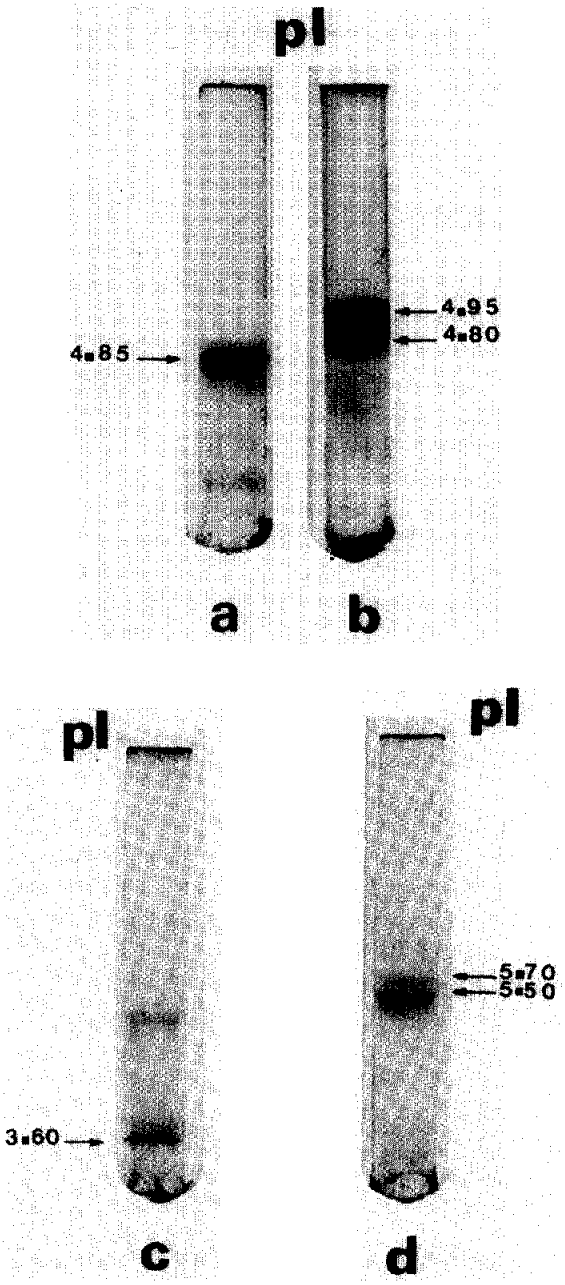

Fig.1. Gel electrofocusing of the proteins studied: (a) αTPI (1) (25 μg sample) pH 3.5–10.0; (b) αTPI (1) (25 μg sample) pH 4.0–6.5; (c) αTPI (2) (25 μg sample) pH 3.5–10.0; (d) cathepsin L (25 μg sample) pH 3.5–10.0.

cathepsin B [15] and is an example of peptidyl-dipeptidase activity. This property has also been found for human liver cathepsin L (table 1), studies with the rat liver enzyme having been reported in [16].

3.2. *Inhibition study*

Cathepsin L activity using azocasein as substrate is strongly inhibited by α-TPI (1) and α-TPI (2) (fig.3).

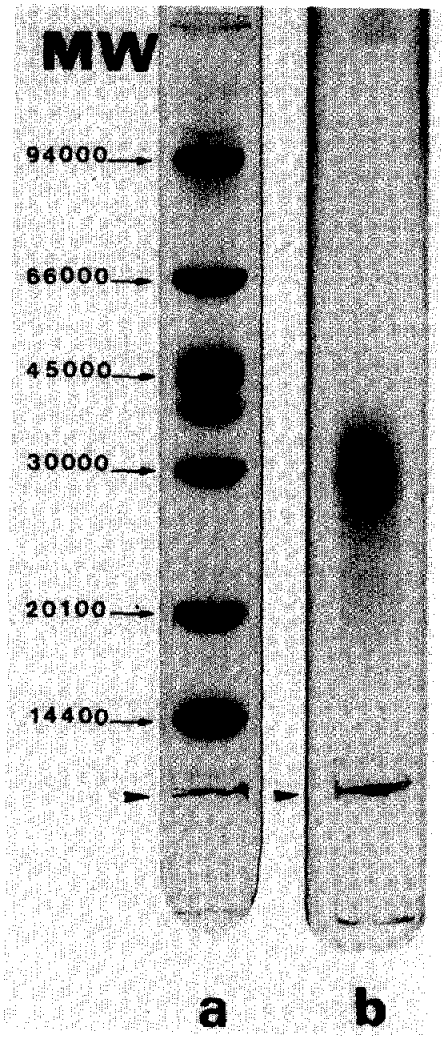

Fig.2. SDS–polyacrylamide gel electrophoresis for cathepsin L (acrylamide 15%, SDS 1%) the sample were run after reduction by dithiotreitol (2%). The arrows at the bottom of the gels show the position of the tracking dye. (a) Mixture of standard proteins: phosphorylase *b* (M_r 94 000); bovine serum albumin (M_r 66 000); ovalbumin (M_r 45 000); carbonic anhydrase (M_r 30 000); soya bean trypsin inhibitor (M_r 20 100); α-lactalbumin (M_r 14 400). (b) Purified cathepsin L (50 μg sample).

Table 1
Some properties of purified human liver cathepsin L

Specific activities (mU/mg at pH 6.0) against:	
BANA	20
Arg-N-Nap	0
Leu-N-Nap	0
Azocasein	150
Aldolase	0.45
$E^{1\%}_{280\ nm,1\ cm}$	11.40
M_r	30 000
Isozymes	
Number	2
pI	5.70
	5.50

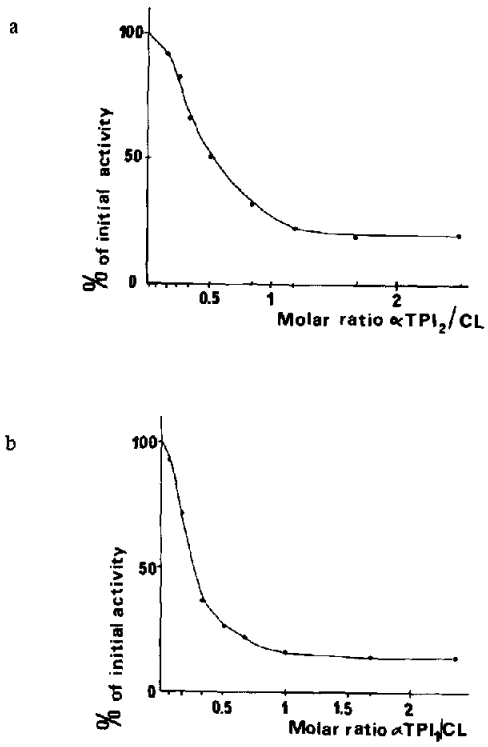

Fig.3. Inhibition curves of cathepsin L by αTPI: (a) αTPI (1); (b) αTPI (2). The enzyme (0.33 nmol) was preincubated for 10 min at room temperature with αTPI (1) (0.02–0.78 nmol) or αTPI (2) (0.01–0.83 nmol) in 400 μl final vol. The substrate (100 μl azocaseine 1%, w/v) was then added and samples were incubated for 2 h at 40°C. Initial cathepsin L activity was determined in the absence of other proteins.

The loss in enzyme activity is 80% with the 2 inhibitors and the inhibition curves show a plateau correlating with 1 binding site on the enzyme.

4. Discussion

Here we describe the purification of human liver cathepsin L and its inhibition by α-TPI (1) and α-TPI (2). Preceding reports on these plasma proteins [1–4] suggest that the inhibitors are group-specific to thiol proteinases, but only plant thiol proteinases are strongly inhibited. The poor inhibition of cathepsin B [2] makes it difficult to understand the physiological function of these inhibitors. Our results show that one of the physiological roles of these plasma proteins is to inhibit cathepsin L. This enzyme has a high proteolytic activity and a very weak activity against synthetic substrate: this fact readily distinguishes cathepsin L from the other 2 thiol proteinases (cathepsin B and cathepsin H) and shows that cathepsin L can play an important role in tissue proteolysis in injury and inflammatory states. The strong inhibition of this enzyme by α-TPI suggests that this protein controls the tissue damage provoked by cathepsin L.

However, it is known that α-TPI accumulates in extracellular fluid, e.g., the pleural cavity and ascites fluid, since here, the protein has been isolated from these fluids. This is in good agreement with the suggested physiological role of these inhibitors.

Acknowledgements

This work was supported by grant CLR 79-1-157-3 from the Institut National de la Santé et de la Recherche Médicale and from the UER Broussais-Hôtel-Dieu, Université Pierre et Marie Curie. The skillful technical assistance of Mrs V. Fumeron is acknowledged.

References

[1] Sasaki, M., Minakata, K., Yamamoto, H., Niwa, M., Kato, T. and Ito, N. (1977) Biochem. Biophys. Res. Commun. 76, 917–924.
[2] Jarvinen, M. (1979) FEBS Lett. 108, 461–464.
[3] Ryley, H. C. (1979) Biochem. Biophys. Res. Commun. 89, 871–878.
[4] Taniguchi, K., Ito, J. and Sasaki, M. (1981) J. Biochem. (Tokyo) 89, 179–184.
[5] Kirschke, H., Langner, J., Wiederanders, B., Ansorge, S. and Bohley, P. (1977) Eur. J. Biochem. 74, 293–301.
[6] Charney, J. and Tomarelli, R. M. (1947) J. Biol. Chem. 171, 501–505.
[7] Pagano, M., Engler, R., Gelin, M. and Jayle, M. F. (1980) Can. J. Biochem. 58, 410–417.
[8] Wrigley, C. (1968) Sci. Tools 15, 2–17.
[9] Davis, B. J. (1964) Ann. N.Y. Acad. Sci. 121, 404–427.
[10] Sasaki, M., Taniguchi, K. and Minakata, K. (1981) J. Biochem. (Tokyo) 89, 169–177.
[11] Etherington, D. J. (1976) Biochem. J. 153, 199–209.
[12] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[13] Schwartz, W. N. and Barrett, A. J. (1980) Biochem. J. 191, 487–497.
[14] Kirschke, H., Langner, J., Wiederanders, B., Ansorge, S., Bohley, P. and Hanson, H. (1977) Acta Biol. Med. Germ. 39, 185–199.
[15] Otto, K. (1971) in: Tissue Proteinases (Barrett, A. J. and Dingle, J. T. eds) pp. 29–38, Elsevier Biomedical, Amsterdam.
[16] Bond, J. S. and Barrett, A. J. (1980) Biochem. J. 189, 17–25.