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Stabilisation of cathepsin E by ATP

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The hydrolysis of 3 distinct substrates by cathepsin E from human red blood cells and gastric mucosa was measured in the presence and absence of physiologically relevant concentrations of ATP. At pH values below about 5.0, the nucleotide was without effect. However, at pH 5.8, whereas cathepsin E was virtually inactive by itself, it was restored to full activity (k_{cat}) by ATP and the non-hydrolysable methylene-ATP analogue. At still higher pH values, k_{cat} progressively diminished but significant levels of cathepsin E activity were readily detectable at pH 7.0. The specificity of this stabilisation effect was examined.

Cathepsin E; Slow moving proteinase; Erythrocyte membrane aspartic proteinase; ATP-stimulation; (Human)

1. INTRODUCTION

Cathepsin E is an aspartic proteinase that has an M_r of around 80000 [1,2], approximately double that of archetypal aspartic proteinases [3]. However, in common with the other enzymes, its catalytic activity (k_{cat}) diminishes significantly at pH values above pH 5.0 [1]. The distribution and physiological function(s) of this proteinase are far from clear. It is present in human gastric mucosa (wherein it has been termed slow moving proteinase [4]) where it has an apparent cytoplasmic localisation by immunohistochemistry [5] and in human erythrocytes where it is associated with the membrane of the cell (and has thus been termed the erythrocyte membrane aspartic proteinase (EMAP) [6]).

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Abbreviations: SMP, slow moving proteinase; EMAP, erythrocyte membrane aspartic proteinase; Nph, nitrophenylalanine

Catabolism of proteins within cells requires ATP (e.g. [7,8]) and particularly within the red blood cell [9,10]. ATP has been reported to have a small activating effect upon the lysosomal aspartic proteinase, cathepsin D [11]. Consequently, it was considered of interest to examine whether ATP had any effect upon cathepsin E (and particularly the enzyme (EMAP) obtained from the red blood cell).

2. MATERIALS AND METHODS

Cathepsin E from human red blood cells (EMAP) and from human gastric mucosa (SMP) and cathepsin D were obtained as described [1,12]. The synthetic chromogenic substrates Lys-Pro-Ile-Glu-Phe-Nph-Arg-Leu and Pro-Pro-Thr-Ile-Phe-Nph-Arg-Leu were synthesised and kinetic parameters (K_m, k_{cat}) for their hydrolysis at a variety of pH values but at constant ionic strength ($\mu = 0.1$ M) were determined as described [13,14]. ATP and other ligands were included at appropriate concentrations when required. [^{3}H]Casein was prepared as described [15].

3. RESULTS AND DISCUSSION

Kinetic parameters for the hydrolysis of Pro-Pro-Thr-Ile-Phe-Nph-Arg-Leu (table 1A) and Lys-

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Table 1

Effect of ATP on the turnover number (k_{cat}) for the hydrolysis of Pro-Pro-Thr-Ile-Phe-Nph-Arg-Leu (A) and Lys-Pro-Ile-Glu-Phe-Nph-Arg-Leu (B) by cathepsin E from erythrocyte membranes at various pH values

pH	k_{cat} (s^{-1})	
	− ATP	+ ATP (6.25 mM)
A		
4.5	36	39
5.0	47	31
5.4	11	39
5.8	~0	40
6.2	~0	16
6.6	~0	11
7.0	~0	<5
B		
4.5	76	77
5.0	54	77
5.4	26	72
5.8	~0	56

Reactions were carried out at 37°C in sodium acetate or MES buffers of the indicated pH value (but with μ always adjusted to 0.1 M by the addition of NaCl as appropriate). The progress of each reaction was monitored at 300 nm. The estimated precision of the values obtained was always in the range of 5–10% (except at pH 7.0)

Pro-Ile-Glu-Phe-Nph-Arg-Leu (table 1B) by cathepsin E from erythrocyte membranes were determined at a variety of pH values. We have reported previously that over the pH range 3–4.5, the rate constants (k_{cat}) for the hydrolysis of these two substrates by cathepsin E are invariant [1]. Consequently, in the present investigation, values for k_{cat} are reported only at pH 4.5 and above. As described previously, above pH 4.5–5.0, k_{cat} values for both substrates diminished substantially so that at pH 5.8, with the amounts of enzyme used for this assay, the rates of substrate cleavage were so low as to render catalysis essentially zero. (The K_m values obtained at all pH values did not appear to alter significantly so that they will not be considered further.)

Inclusion of ATP in the assays at a concentration (6.25 mM) approximating that within cells had no effect on the k_{cat} values of the enzyme with either substrate at low pH values (e.g. pH 4.5). By contrast, as the pH was raised above pH 5.0, a dramatic stabilisation of the enzyme was observed such that at pH 5.8, virtually full expression of the catalytic efficiency of the enzyme was observed in the presence of ATP. As the pH was raised even higher, the k_{cat} values measured against Pro-Pro-Thr-Ile-Phe-Nph-Arg-Leu began to diminish steadily (table 1A) but, nevertheless, significant levels of the aspartic proteinase activity were still readily detectable at pH 7.0. Unfortunately, the molar extinction change observed upon cleavage of the Phe-Nph bond in the substrate becomes progressively smaller with rising pH, making precise measurements increasingly more difficult to obtain at the higher pH values (especially at pH 7.0). Considerable amounts of enzyme have to be added so that these experiments were not repeated at the highest pH values with the Lys-Pro-Ile-Glu-Phe-Nph-Arg-Leu substrate. However, with the Pro-Pro-Thr-Ile-Phe-Nph-Arg-Leu substrate (table 1A), the extent of cleavage (e.g. at pH 7.0) was monitored in a different manner by observing the appearance of the expected hydrolysis fragments (Pro-Pro-Thr-Ile-Phe and Nph-Arg-Leu) by reversed phase FPLC (not shown). From the rates thus obtained, a k_{cat} value was estimated which was consistent with the approximate value included in table 1A (from the spectrophotometric measurements).

Since the maximum stabilisation effect was observed at pH 5.8, this pH was selected for further investigations. That the effect was unrelated to the substrate was demonstrated by the identical results obtained with the two different chromogenic peptide substrates and also when [^{3}H]casein was utilised as a protein substrate. For the latter, the activities measured at pH 5.8 were 600 cpm/h per μg enzyme (without ATP) and 20000 cpm/h per μg (+ 6.25 mM ATP), respectively. At pH 5.0, a much smaller difference was observed between the activities measured without (16000) and with 6.25 mM ATP (42000 cpm/h per μg), just as observed with the peptide substrates.

The affinity constant of ATP for the erythrocyte membrane cathepsin E was 15 mM (fig.1).

In order to determine the specificity of this stabilisation effect, the influences of other ligands on the enzyme were measured at pH 5.8. Adenosine, (sodium)triphosphate (at a concentration which produced an ionic strength equivalent to that obtained with 6.25 mM ATP – to ensure that this effect was not produced by an altered

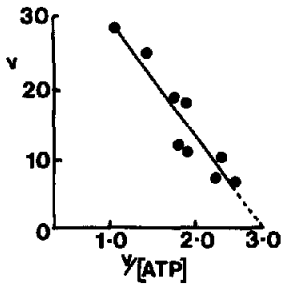

Fig.1. Determination of the binding constant of ATP at pH 5.8 for cathepsin E from erythrocyte membranes. ATP was introduced at increasing concentrations into reaction cuvettes containing cathepsin E and the peptide substrate, Lys-Pro-Ile-Glu-Phe-Nph-Arg-Leu in 0.1 M sodium acetate buffer, pH 5.8. Reaction rates (v) were determined from the decrease in absorbance at 300 nm.

ionic strength), ADP and 2,3-diphosphoglycerate had no effect on the enzyme whatsoever (table 2). By contrast, the non-hydrolysable β-γ-CH_2-ATP analogue produced an equivalent stabilisation to that obtained with ATP.

Mercaptoethanol, magnesium or vanadate ions did not alter the effect obtained with ATP alone and all were without effect when added alone. GTP and CTP also brought about a stabilisation in the activity of the enzyme (table 2).

Previously, we have shown that cathepsin E from erythrocyte membranes is indistinguishable from the slow moving (aspartic) proteinase from gastric mucosa [1]. In further confirmation of this, directly comparable results were obtained with ATP for the restoration of the slow moving proteinase to full activity at pH values above 5.0 (not shown).

Table 2

Effect of various ligands on the catalytic rate constant (k_{cat}) for the hydrolysis of Lys-Pro-Ile-Glu-Phe-Nph-Arg-Leu at pH 5.8 by the cathepsin E from erythrocyte membranes

Ligand	Concentration (mM)	k_{cat} (s^{-1})
None	6.25	~0
Adenosine	6.25	~0
'Tri-phosphate'	2.5	~0
ADP	6.25	~0
ATP	6.25	56
β-γ-CH_2-ATP	6.25	54
GTP	6.25	18
CTP	6.25	39
2,3 DPG	10.0	~0

By contrast, cathepsin D (of human or rat origin) was unaffected by ATP when assayed against either of the two synthetic substrates. It was inactive at pH 5.8 in the absence of the ATP and inclusion of the nucleotide (to concentrations up to 15 mM) did not elevate the activity to measurable levels. When [^{3}H]casein was used as a substrate, the activities measured for cathepsin D were 6500 and 11000 cpm/h per μg at pH 4.5 and 3800 and 7200 at pH 5.8 in the absence and presence of (6.25 mM) ATP, respectively. Thus, the effect of ATP on cathepsin D is very small compared to that observed with cathepsin E and is clearly related to the nature of the substrate employed [11].

No information is available on the primary or tertiary structure of cathepsin E to facilitate molecular interpretation of this ATP effect. In the absence of ATP, the loss of activity observed above pH 5.0 is not due to autolysis of the enzyme but rather reflects the loss of one or more protons from the catalytic apparatus [1,14]. Thus, aspartic proteinases (or carboxyl or acid proteinases as they were formerly termed) are commonly considered to be constrained to operating under acidic conditions. However, as the present findings indicate, the presence of a ligand such as ATP can influence the expression of the cathepsin E activity considerably. The nucleotide does not appear to be activating the proteinase since the turnover numbers measured at pH values below 5.0 in the presence of ATP are not significantly increased over those determined in its absence (table 1). Furthermore, the inclusion of ATP at pH 5.8 restored the turnover number (table 1A) back only to the magnitude measured at the lower pH values. It might then be considered that ATP acts (in a long-range effect) in such a way as to maintain the enzyme in its active conformation, thus suppressing dissociation of the proton(s) from the catalytic residues and prolonging the expression of the intrinsic activity to higher pH values. Since this aspartic proteinase appears to have an intracellular (but non-lysosomal) localisation [5,6], it would seem worthy of further examination to investigate whether this enzyme can be provoked by ATP into playing a part in intracellular protein processing or breakdown.

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REFERENCES

[1] Jupp, R.A., Richards, A.D., Kay, J., Dunn, B.M., Wyckoff, J.B., Samloff, I.M. and Yamamoto, K. (1988) Biochem. J. 254, 895–898.
[2] Yamamoto, K., Katsuda, N. and Kato, K. (1978) Eur. J. Biochem. 92, 499–508.
[3] Kay, J., Jupp, R.A., Norey, C.G., Richards, A.D., Reid, W.A., Taggart, R.T., Samloff, I.M. and Dunn, B.M. (1988) in: Proteinases: Potential Role in Health and Disease (Heidland, A. and Horl, W.H. eds) Plenum, New York, in press.
[4] Samloff, I.M., Taggart, R.T., Shiraishi, T., Branch, T., Reid, W.A., Heath, R., Lewis, R.W., Valler, M.J. and Kay, J. (1987) Gastroenterology 93, 77–84.
[5] Kay, J., Thomas, D.J., Richards, A.D., Jupp, R.A., Reid, W.A., Samloff, I.M., Dunn, B.M. and Yamamoto, K. (1988) in: Intracellular Protein Catabolism VII (Katunuma, N. and Kominami, E. eds) Springer, Berlin, in press.
[6] Yamamoto, K., Ueno, E., Uemura, H. and Kato, Y. (1987) Biochem. Biophys. Res. Commun. 148, 267–272.
[7] Beynon, R.J. and Bond, J.S. (1986) Am. J. Physiol. 251, C141–152.
[8] Mayer, R.J. and Doherty, F. (1986) FEBS Lett. 198, 181–193.
[9] Tanaka, A., Waxman, L. and Goldberg, A.L. (1983) J. Cell Biol. 96, 1580–1585.
[10] Hough, R., Pratt, G. and Rechsteiner, M. (1986) J. Biol. Chem. 261, 2400–2408.
[11] Watabe, S., Terada, A., Ikeda, T., Kouyama, H., Taguchi, S. and Yago, N. (1979) Biochem. Biophys. Res. Commun. 89, 1161–1167; Pillai, S., Botti, R. and Zull, J.E. (1983) J. Biol. Chem. 258, 9724–9728.
[12] Bonelli, G., Kay, J., Tessitore, L., Jupp, R.A., Isidoro, C., Norey, C.G., Autelli, R., Richards, A.D. and Baccino, F.M. (1988) Z. Physiol. Chem. (Hoppe-Seyler) 369, 323–327.
[13] Valler, M.J., Kay, J., Aoyagi, T. and Dunn, B.M. (1985) J. Enzyme Inhib. 1, 77–82.
[14] Dunn, B.M., Valler, M.J., Rolph, C.E., Foundling, S.I., Jimenez, M. and Kay, J. (1987) Biochim. Biophys. Acta 913, 122–130.
[15] Jentoft, N. and Dearborn, D.G. (1979) J. Biol. Chem. 254, 4359–4365.