Action of Human Cathepsin G on the Oxidized B Chain of Insulin

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The specificity of cathepsin G, a serine neutral proteinase from human neutrophil leucocytes, was determined by its action on the insulin B chain. The most susceptible bonds were Phe-24–Phe-25, Leu-15–Tyr-16 and Tyr-16–Leu-17. Other bonds hydrolysed were Leu-6–Cys(O₃H)-7, Leu-11–Val-12, Leu-17–Val-18 and Phe-25–Tyr-26. These results suggest that the specificity of cathepsin G is closer to that of pig chymotrypsin C than ox chymotrypsin A. Tables listing amino acid composition, *N*-terminal residue, and yields of isolated peptides have been deposited as Supplementary Publication SUP 50075 (8 pages) at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7B2, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1977) 161, 1.

Cathepsin G, a serine neutral proteinase purified from human spleen (Starkey & Barrett, 1976a), has been shown to be immunologically identical with the 'chymotrypsin-like' enzyme of azurophil granules of neutrophil granulocytes (Starkey & Barrett, 1976b). The enzyme hydrolyses several synthetic substrates also hydrolysed by ox chymotrypsin A, but with different kinetic constants, and its sensitivity to inhibitors of chymotrypsin A is also different. In the present study we investigated the action of cathepsin G on a polypeptide substrate, the oxidized B chain of insulin, and compared it with that described for ox chymotrypsin A (EC 3.4.21.1; α -chymotrypsin; Sanger & Tuppy, 1951) and pig chymotrypsin C (EC 3.4.21.2; Folk & Cole, 1965).

Experimental

Materials

Sephadex G-25 (fine grade) was from Pharmacia (G.B.), London W.5, U.K. Other materials were as listed in the preceding paper (Blow, 1977).

Methods

Separation of digestion products of insulin B chain. Oxidized B chain (15 mg in 1 ml of 0.1 M-NH₄HCO₃, pH 8.5) was digested with 75 μ g of cathepsin G for 30 min or 4 h at 37°C. The reaction was stopped by lowering the pH to 3.0 with 6 M-HCl. The digests were fractionated on a Sephadex G-25 column (110 cm $\times$ 1 cm), equilibrated and eluted with 0.1 M-NH₄HCO₃,

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as well as on an ion-exchange resin, as described by Blow (1977). Other methods were as described by Blow (1977).

Results

Digestion for 4 h

Preliminary experiments in which products were separated by high-voltage electrophoresis indicated that, after 4 h of digestion, no undigested B chain remained. When oxidized insulin B chain was digested for 4 h and fractionated by Sephadex G-25 column chromatography, the *A*₂₂₀ trace indicated that individual peptides had not been well resolved, so high-voltage electrophoresis was used to indicate which fractions should be pooled and concentrated. From these pooled fractions, individual components were purified by preparative high-voltage electrophoresis. Four major products were isolated, and after dansylation and amino acid analysis these could be identified as peptides containing residues 1–15 (21% yield), 16–24 (5.2% yield), 25–30 (6.5% yield) and 16–17 (3.8% yield), accounting for all the residues in the B chain.

The results indicate a major site of action for cathepsin G between leucine-15 and tyrosine-16, which is a minor site for chymotrypsin A (Sanger & Tuppy, 1951), but a major site for chymotrypsin C (Folk & Cole, 1965). There is no evidence from these data for a cleavage between tyrosine-16 and leucine-17 (a major site for chymotrypsin A, but a minor site for chymotrypsin C). This pattern is repeated at the other major cleavage site, phenylalanine-24 to tyrosine-26. Cathepsin G, in common with chymotrypsin C, cleaves between phenylalanine-24 and phenylalanine-25. This is not a site for chymotrypsin A. Both

chymotrypsins A and C, and to a minor extent cathepsin G (see under 'Fractionation by ion-exchange resin'), cleave between phenylalanine-25 and tyrosine-26. The other major chymotrypsin-A site, between tyrosine-26 and threonine-27, is not attacked by cathepsin G, but it is, to a minor extent, by chymotrypsin C. A minor site for cathepsin G is indicated between leucine-17 and valine-18, which is a major site for chymotrypsin C, though not cleaved by chymotrypsin A. The results can be summarized by saying that cathepsin G shows more similarity to chymotrypsin C than to chymotrypsin A; peptide bonds involving the carboxyl group of leucine and phenylalanine are cleaved, but not tyrosine (see Fig. 1). Chymotrypsin C, however, cleaves more bonds than does cathepsin G.

Digestion for 30 min

A 30 min digest, fractionated in the same way, gave only one major product, and four minor ones. The major peptide contained residues 1-15 (20% yield), and the minor peptides, all recovered in less than 2% yield, were peptides containing residues 16-30, 25-30 and mixtures probably consisting of peptides containing residues 26-30, 16-17 and free phenylalanine and alanine. The free phenylalanine could have come from the peptide containing residues 25-30, since the product containing residues 26-30 was also recovered, but the origin of the free alanine is unknown. Indicated cleavage sites from these data are the same as found in the preceding section, and yields, though these are not very reliable for a method that relies so heavily on recovery after paper electrophoresis, appear to indicate that the first site of action is between leucine-15 and tyrosine-16.

Fractionation by ion-exchange resin

To obtain more conclusive evidence on the relative importance of the various sites of action of cathepsin G, an ion-exchange resin fractionation, as successfully used for a similar project for lysosomal elastase (Blow, 1977), was done for a 4 h digest on insulin B chain by cathepsin G. High-voltage electrophoresis showed that most of the separated fractions contained several components, and these were isolated by preparative high-voltage electrophoresis.

Four major peptides were found in high yield, comprising residues 25-30 (26.4% yield), 17-24 (16.2% yield), 16-24 (15.7% yield) and 1-15 (15.3% yield), accounting for the complete sequence of the B chain, and three of these are the three major peptides found after Sephadex G-25 chromatography. This confirms that major sites of cleavage are between leucine-15 and tyrosine-16 and between phenylalanine-24 and phenylalanine-25, and suggests that there is another relatively major site between tyrosine-

Fig. 1. Specificity of action of human cathepsin G (a), pig chymotrypsin C (b) and ox chymotrypsin A (c) on insulin B chain. Large arrows, major sites; small arrows, minor sites. Data for (b) and (c) are from Folk & Cole (1965) and Sanger & Tuppy (1951) respectively. The digestion conditions and purification of the digestion products were as described in the text.

16 and leucine-17. Thus cleavage at the large hydrophobic residues of leucine, phenylalanine and tyrosine appears to be favoured, particularly when they are present in an extended hydrophobic site.

Minor peptides comprising residues 16-17, 16-25 and 26-30 (all in 3-4% yield) again indicate minor cleavage sites between leucine-17 and valine-18 and between phenylalanine-25 and tyrosine-26. These sites make interesting comparisons with the three listed above, as they are superficially quite similar.

and

Peptide containing residues 7-15 (8% yield) indicates a minor cleavage at another leucine residue, between leucine-6 and cysteine-7, or, in extended form

which is a site for chymotrypsin C, but not for chymotrypsin A. There is also evidence [peptides containing residues 1-11 (4% yield) and 12-15 (2% yield)] for a minor cleavage at the similar site

which is another site for chymotrypsin C.

Discussion

In its action on the oxidized B chain of insulin, cathepsin G more closely resembles pig chymotrypsin C than ox chymotrypsin A. The latter enzyme is much more specific than either cathepsin G or chymotrypsin C, cleaving the chain at only one of the four available leucine residues, and at only one of the three available phenylalanine residues. The three enzymes are similar, however, in that cleavages only occur adjacent to a phenylalanine, tyrosine or leucine residue (chymotrypsin C also cleaves at a glutamine residue), whereas by contrast the elastases (Blow, 1977) cleave next to valine and alanine residues.

This variation in the sites cleaved by individual 'chymotrypsin-like' enzymes makes the term only of limited use in describing the specificity of such enzymes.

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