Vol. 93, No. 2, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 28, 1980 Pages 399-402

ACTIVE SITE OF α_1-ANTITRYPSIN: HOMOLOGOUS SITE IN ANTITHROMBIN-III

Robin W. Carrell, D. Ross Boswell, Stephen O. Brennan & Maurice C. Owen

Molecular Pathology Laboratory, Pathology Department,
Christchurch Hospital, New Zealand

Received February 1,1980

SUMMARY: Examination of peptides resulting from reaction of bovine trypsin and human α_1-antitrypsin in near-equimolar amounts showed anomalous cleavage of antitrypsin at a Met-Ser bond 37 residues from the C-terminus, giving evidence that this is the active site for trypsin inhibition. Alignment of the C-terminal 141 residues of α_1-antitrypsin with the C-terminal 147 residues of human antithrombin-III showed homology with 30% identity and allowed the identification of a homologous active site in antithrombin.

INTRODUCTION

In an earlier paper (1) the sequence of the C-terminal third of human α_1-antitrypsin was presented and it was noted that it is similar to the C-terminus of human antithrombin-III. It was also noted that there was partial cleavage at a Met-Ser bond 37 residues from the C-terminus of antitrypsin by catalytic amounts of trypsin. We now present results of further investigations of these observations.

METHODS

Tryptic cleavage of α_1-antitrypsin

Human α_1-antitrypsin was isolated from plasma as previously described (2). Antitrypsin, 52mg, was dissolved in 2ml of 25 mM Tris/HCl, pH 7.3; to this was added 17 mg of twice-crystallised bovine trypsin (Sigma) dissolved in 1 ml of buffer. The solutions were mixed on a vortex mixer for 30 sec prior to the addition of 5 µl of formic acid and 7 ml of 95% ethanol. The protein precipitate was separated and shown by sodium dodecyl sulphate polyacrylamide gel electrophoresis to consist primarily of the protease-antiprotease complex with a lesser amount of unreacted antitrypsin. The ethanol supernatant was dried *in vacuo* and subjected to peptide mapping essentially as described in (2). Peptides were eluted from preparative maps and were characterised by amino acid analysis. Their amino acid sequences were determined by the dansyl Edman procedure (3).

Homology of α_1-antitrypsin and antithrombin-III

A maximal match of the C-terminal 141 residues of α_1-antitrypsin (1) with the C-terminal 147 residues of antithrombin-III (4) was performed using the algorithm of Needleman & Wunsch (5) with the MDM_{78} score matrix of Schwartz & Dayhoff (6). Matrix bias and gap penalty parameters of 60 & 100

0006-291X/80/060399-04$01.00/0

respectively were determined empirically. The alignment score was converted to a standard score by comparison with the distribution of scores obtained for 100 different randomisations generated by selection from the real sequences with replacement.

RESULTS AND DISCUSSION

Tryptic cleavage of α_1-antitrypsin

Three of the four expected tryptic peptides from the C-terminal 36 residues of antitrypsin were identified (Fig. 1). Three further minor peptides were shown to be tryptic fragments of trypsin. No peptides from antitrypsin proximal to the C-terminal 36 residues were identified. Our interpretation of these data is that the small fragment known to be released in the formation of the trypsin-antitrypsin complex (7) is the C-terminal 36-residue portion of antitrypsin, and that following its release under these conditions it is subjected to further tryptic cleavage. Corroborative evidence for this has been provided recently by Morii and others (8) who have determined the N-terminal sequence (5 residues) and the C-terminal sequence (2 residues) of the fragment released from antitrypsin by bovine α-chymotrypsin, and have estimated its

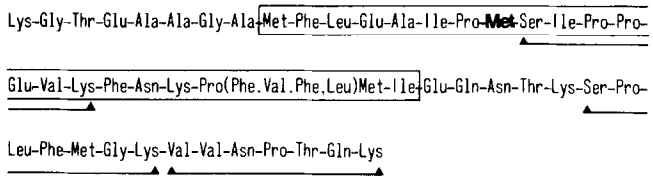

Fig. 1 The C-terminal sequence of human α_1-antitrypsin (1). The active centre Met (bold faced) is embedded in a sequence 25 residues long (boxed) identical to that proposed by others (10,11) for an N-terminal active site. The underscores indicate peptides identified after reaction of antitrypsin and trypsin in near-equimolar amounts.

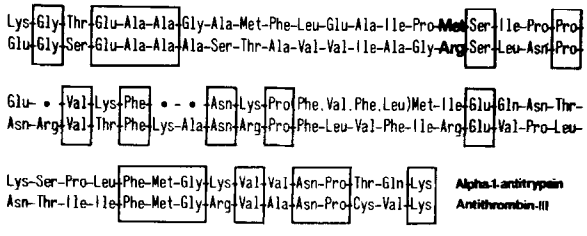

Fig. 2 The C-terminal portion of the alignment of human α_1-antitrypsin (1) with human antithrombin-III (4). The active centre residues are shown in bold face. Boxes indicate conserved amino acids.

-Met-Ser-Ile-Pro-Pro-Glu-	Alpha-1-antitrypsin
-Arg-Ser-Leu-Asn-Pro-Asn-	Human antithrombin-III
(Arg)Ser-Leu-Asn-Ser-Asp-	Bovine antithrombin-III
-Ala-Ser-Ile-Pro-Pro-Gln-	Garden bean
-Arg-Ser-Asx-Pro-Pro-Glx-	Peanut

Fig. 3 The proposed homologous active sites of human α_1-antitrypsin and human antithrombin-III are shown for comparison with the thrombin cleavage site in bovine antithrombin-III (9) and the first reactive sites of II-garden bean and peanut protease inhibitors (12).

molecular weight as 3600 daltons. These results are consistent with cleavage at the Met 37 residues from the C-terminus (Fig. 1).

Homology of α_1-antitrypsin and antithrombin-III

The alignment (Fig. 2) confirmed earlier impressions of homology, giving a standard score of 11.9. This indicates a probability less than 10^{-23} that the observed degree of similarity could have arisen by chance alone from sequences of these compositions. The proportion of identical residues in the alignment is 30%. The reactive Met in antitrypsin is aligned with Arg-384 in antithrombin and the sequences following these residues are similar to those of other serine protease inhibitors (Fig. 3). This reactive site prediction has recently been supported by the finding of Jornvall and others (9) of a thrombin cleavage site in bovine antithrombin homologous with the Arg-384 site in human antithrombin.

There is some confusion in the literature over the placement of the active site of α_1-antitrypsin. Others (10,11) have described a Met active centre embedded in a sequence 25 residues long identical to that which we find (Fig. 1), but have placed it near the N-terminus of antitrypsin. The findings we now report, taken together, provide strong evidence for a C-terminal placement of the active site.

ACKNOWLEDGEMENTS

We gratefully acknowledge the stimulus and help received from Dr A.D.McLachlan, MRC Laboratory of Molecular Biology, Cambridge.

This work was supported by the Medical Research Council of New Zealand.

REFERENCES

1. Carrell, R.W., Owen, M.C., Brennan, S.O. and Vaughan, L. (1979) Biochem. Biophys. Res. Commun. 91, 1032-1037.
2. Owen, M.C., Carrell, R.W. and Brennan, S.O. (1976) Biochim. Biophys. Acta 453, 257-261.
3. Hartley, B.S. (1970) Biochem. J. 119, 805-822.
4. Petersen, T.E., Dudek-Wojciechowska, G., Sottrup-Jensen, L. and Magnusson, S. (1978) Atlas of Protein Sequence and Structure, Vol. 5 (ed. Dayhoff, M.O.) p.141, National Biomedical Research Foundation, Washington.
5. Needleman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol. 48, 443-453.
6. Schwartz, R.M. and Dayhoff, M.O. (1978) Atlas of Protein Sequence and Structure, Vol. 5 (ed. Dayhoff, M.O.) p.354, National Biomedical Research Foundation, Washington.
7. Moroi, M. and Yamasaki, M. (1974) Biochim. Biophys. Acta 359, 130-141.
8. Morii, M., Odani, S. and Ikenaka, T. (1979) J. Biochem. 86, 915-921.
9. Jornvall, H., Fish, W.W. and Bjork, I. (1979) FEBS Lett. 106, 358-363.
10. Johnson, D. and Travis, J. (1978) J. Biol. Chem. 253, 7142-7144.
11. Kress, L.F., Kurecki, T., Chan, S.K. and Laskowski, M. (1979) J. Biol. Chem. 254, 5317-5320.
12. Ketcham, L.K., Barker, W.C. and Dayhoff, M.O. (1978) Atlas of Protein Sequence and Structure, Vol. 5 (ed. Dayhoff, M.O.) p. 136, National Biomedical Research Foundation, Washington.