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The reactive serine residue of epidermolytic toxin A

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Comparison of amino acid sequence data suggested that there may be a functional relationship between the staphylococcal epidermolytic toxins and V8 proteinase. The hypothesis was tested by treating epidermolytic toxin with di-isopropyl phosphorofluoridate, which bound specifically at serine-195, the homologue of the active-site serine residue of V8 proteinase.

INTRODUCTION

Epidermolytic toxins (ETs) are staphylococcal proteins that cause skin-splitting in man and other mammals [1]. The nucleotide sequences for the two serotypes ETA and ETB have been established by three groups [2–4]. No evidence of a homologous relationship between the toxins and any other proteins was noted by these workers. However, recent issues of standard sequence-data bases include results for the V8 proteinase of *Staphylococcus aureus* [5], and in a routine re-examination we have now found that the amino acid sequences of epidermolytic toxins have a marked resemblance to that of the V8 proteinase, originally described by Drapeau [6] in 1978.

We have therefore carried out experiments to determine whether ET has any of the properties expected of a serine proteinase.

EXPERIMENTAL

ETA was purified from culture supernatants of an overproducing strain of *Staphylococcus aureus* [7] by isoelectric focusing and gel filtration [8]. Di-[1,3-^{3}H]isopropyl phosphorofluoridate ([1,3-^{3}H]iPr_2*P*-F) was from NEN Research Products. Unlabelled iPr_2*P*-F and Tos-Phe-Ch_2Cl-treated trypsin were from Sigma Chemical Co.

The toxin (2 mg/ml) was radiolabelled by incubation at 37 °C in 0.14 M-NaCl/10 mM-sodium phosphate buffer, pH 7.4, for 1 h with [1,3-^{3}H]iPr_2*P*-F (3 Ci/mmol; 20 μCi/ml). Unlabelled iPr_2*P*-F was added to 5 mM, and the incubation was continued for a further 1 h.

Tryptic peptides were separated by reverse-phase chromatography on Aquapore RP-300 columns (30 mm × 2.1 mm) in acetonitrile gradients and detected by absorption at 208 nm in an LKB Ultrospec instrument. Amino acid sequencing was carried out by using the automated Edman procedure of the Applied Biosystems 477A apparatus. Epidermolytic activity was assessed by the end-point assay procedure *in vitro* [9].

RESULTS AND DISCUSSION

A 6% fraction of the total radioactivity was incorporated into trichloroacetic acid-insoluble protein during the radiolabelling reaction. The protein product was recovered after chromatography on Sephadex G-25. Tryptic digests (enzyme/substrate weight ratio 1:100 in 0.2 M-NH_4HCO_3, for 3 h at 37 °C, repeated once) of the labelled protein were separated by reverse-phase chromatography (Fig. 1). Over 80% of the recovered radioactivity was found in one fraction. Sequence determination showed that the radioactive fraction contained a tryptic fragment corresponding to residues 186–213 (plus a fragment that contained no serine and corresponded to residues 109–125). The radioactivity of the fragment was released only in cycle 10 of the Edman degradation (Fig. 2). This established that the radioactive peptide has the *N*-terminal sequence YYGFTVPGNS*GSGIFNS. Notably cycles 12 and 17, which also yielded serine residues, were

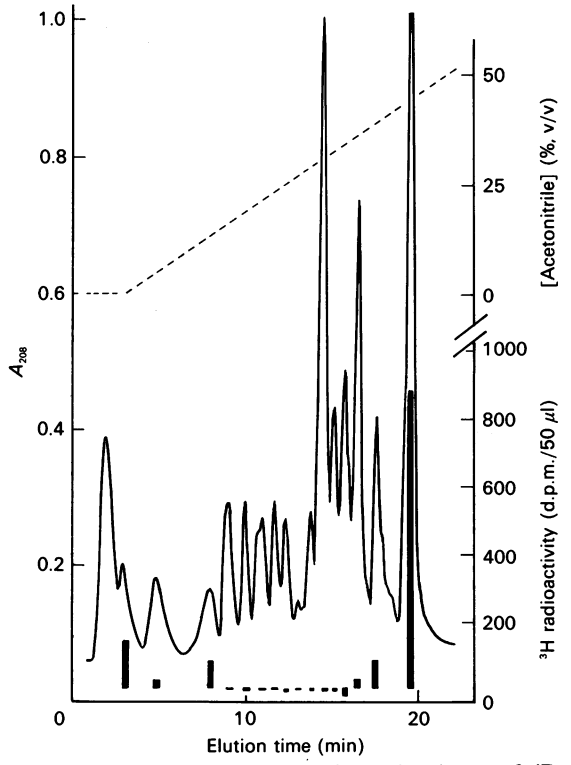

Fig. 1. Reverse-phase chromatography of tryptic digests of iPr_2*P*-F-labelled ETA

The digest (100 μg) was separated at a flow rate of 0.2 ml/min by chromatography in a linear gradient of acetonitrile (------) in 0.1% (v/v) trifluoroacetic acid and detected spectrophotometrically (——). Samples of the separated peptides were taken for ^{3}H determination by liquid-scintillation counting (histogram).

Abbreviations used: ET, epidermolytic toxin; ETA and ETB, epidermolytic toxin serotypes A and B respectively; iPr_2*P*-F, di-isopropyl phosphorofluoridate.

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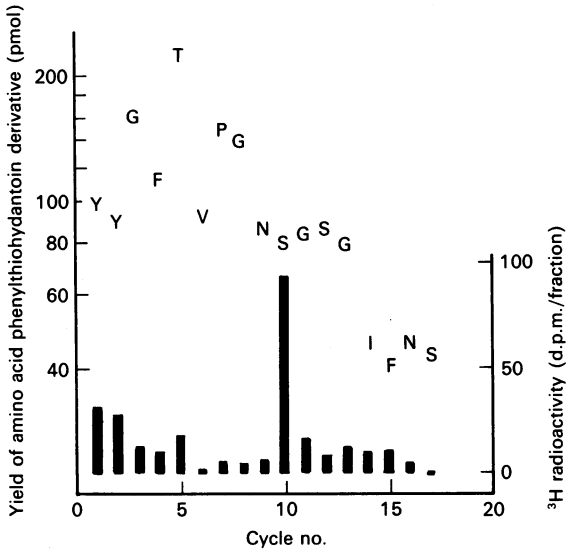

Fig. 2. Sequence of the radiolabelled peptide of ETA

The yield of the identified amino acid is indicated by the placing of the one-letter names. The radioactivity recovered in the fractions is indicated by the bar presentation.

not accompanied by release of radiolabel. Reaction with iPr_2P-F is a classical test [10] of serine-proteinase reactivity, and V8 proteinase is completely inactivated [11], with quantitative reaction at the active-site serine-169 [6], under conditions similar to those of the present experiment.

The sequence similarity of ETA, ETB and V8 proteinase is shown in Fig. 3, as aligned by the Clustal procedure [12]. There is about 25% identity between ETA and V8 proteinase, which is only moderate evidence for a homologous relationship. However, the concentration of conserved sequence, at and near the residues corresponding to the active-site histidine-51, aspartic acid-93 and serine-169, strengthens the hypothesis that ET may be functionally related to V8 proteinase.

The demonstration that ETA will specifically bind iPr_2P-F at serine-195, the exact homologue of the active-site serine-169 of V8 proteinase, supports the hypothesis of a functional relationship. However, it is well established that ET has no proteolytic activity against a range of substrates [13–15]. In new tests we have been unable to detect any proteolytic activity using a sensitive fluorescence assay [16].

The iPr_2P-F-treated protein obtained after Sephadex chromatography was active at 50 μg/ml in the end-point assay *in vitro* [9]. The direct assay of epidermolysis is a crude test that can only detect losses in activity of 50% or more. From the yield at cycle 10 of the sequence determination (Fig. 2) it is clear that serine-195 has not reacted completely, and it may be that any loss of activity accompanying reaction with iPr_2P-F was too small to be detected by our procedure.

The evidence that ET has serine-proteinase-like properties and binds to the skin protein profilaggrin [17–19] presents two clues to the mechanism of action of the toxin.

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                    .         .         .         .         .         .
ETA       1   -EVSAEEIKKHEEKWNKYYGVNAFNLPKELFSKVDEKDRQKYPYNTIGNVFVKGQTSATG  59
ETB       1   KEYSAEEIRKLKQK----FEVPPTD--KELYTHITDNARS--PYNSVGTVFVKGSTLATG  52
V8        1   VILPNNDRHQITDTTNGHYAPVT-------YIQVEAPT----------GTFI-----ASG  38
                 :::: :: :::    ::  :       : :::: :          ::*:     *:*

                    .         .         .         .         .         .
ETA      60   VLIGKNTVLTNRHIAKFANGDPSKV-SFRPSINTD-DNGNTETPYGEYEVKEILQEPFGA 117
ETB      53   VLIGKNTIVTNYHVAREAAKNPSNI-IFTPAQNRDAEKNEFPTPYGKFEAEEIKESPYGQ 111
V8       39   VVVGKDTLLTNKHVVDATHGDPHALKAFPSAINQDN------YPNGGFTAEQITK--YSG  90
              *::**:*::** *::  :  :*  :  * :: * *       * * ::::::* :  ::
                          *

                    .         .         .         .         .         .
ETA     118   GVDLALIRLKPDQNGVSLGDKISPAKIGTSNDLKDGDKLELIGYPFDHKVNQMHRSEIEL 177
ETB     112   GLDLAIIKLKPNEKGESAGDLIQPANIPDHIDIQKGDKYSLLGYPYNYSAYSLYQSQIEM 171
V8       91   EGDLAIVKFSPNEQNKHIGEVVKPATMSNNAETQVNQNITVTGYPGDKPVATMWESKGKI 150
              : ***:::::*::::   *: : **:: :  : : ::: :: *** :  :  :  *: ::
                *

                    .         .         .         .         .         .
ETA     178   TTL-SRGLRYYGFTVPGNSGSGIFNSNGELVGIHSSKVSH--------LDREHQ------ 222
ETB     172   FND-S---QYFGYTEVGNSGSGIFNLKGELIGIHSGKGGQHNLPIGVFFNRKIS------ 221
V8      151   TYLKGEAMQYDLSTTGGNSGSPVFNEKNEVIGIHWG-GVPNEFNGAVFINENVRNFLKQN 209
                 :   :*   *  ***** :** ::*::*** :   :        :: :
                          *

                    .         .         .         .         .         .
ETA     223   ---INYGVG--IGNYVKRIINEK---NE---------------------------- 242
ETB     222   ---SLYSVDNTFGDTLGNDLKKRAKLDK---------------------------- 246
V8      210   IEDIHFANDDQPNNPDNPDNPNNPDNPNNPDEPNNPDNPNNPDNPDNGDNNNSDNPDAA 268
                 :: :   ::        ::    :
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Fig. 3. Clustal alignment of the amino acid sequences of ETA, ETB and *Staphylococcus aureus* V8 proteinase

The locations of matching residues (*), conservative substitutions (:) and the histidine, aspartic acid and serine active-site residues (⁂) are indicated.

REFERENCES

1. Freer, J. H. & Arbuthnott, J. P. (1983) Pharmacol. Ther. **19**, 55–106
2. Lee, C. Y., Schmidt, J. J., Johnson-Winegar, A. D., Spero, L. & Iandolo, J. J. (1987) J. Bacteriol. **169**, 3904–3909
3. O'Toole, P. W. & Foster, T. J. (1987) J. Bacteriol. **169**, 3910–3915
4. Sakurai, S., Suzuke, H. & Kondo, I. (1988) J. Gen. Microbiol. **134**, 711–717
5. Carmona, C. & Gray, G. L. (1987) Nucleic Acids Res. **15**, 6757
6. Drapeau, G. R. (1978) Can. J. Biochem. **56**, 534–544
7. O'Toole, P. W. (1987) Ph.D. Thesis, University of Dublin
8. Bailey, C. J., Martin, S. M. & Bayley, P. M. (1983) Biochem. J. **203**, 775–778
9. Smith, T. P. & Bailey, C. J. (1990) Toxicon, in the press
10. Kraut, J. (1977) Annu. Rev. Biochem. **46**, 331–358
11. Drapeau, G. R., Boily, Y. & Hoummard, J. (1972) J. Biol. Chem. **247**, 6720–6726
12. Higgins, D. & Sharpe, P. M. (1988) Gene **73**, 237–244
13. Arbuthnott, J. P., Billcliffe, B. & Thompson, W. D. (1974) FEBS Lett. **46**, 92–95
14. Rogolsky, M., Wiley, B. B., Keyhani, M. & Glasgow, L. A. (1974) Infect. Immun. **10**, 1261–1265
15. Bailey, C. J., De Azavedo, J. & Arbuthnott, J. P. (1980) Biochim. Biophys. Acta **624**, 111–120
16. Twining, S. S. (1984) Anal. Biochem. **143**, 30–34
17. Smith, T. P. & Bailey, C. J. (1986) FEBS Lett. **194**, 309–312
18. Smith, T. P., John, D. A. & Bailey, C. J. (1987) Histochem. J. **19**, 137–149
19. Smith, T. P., John, D. A. & Bailey, C. J. (1989) Eur. J. Cell Biol. **49**, 341–349

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