Epidermolytic toxin from *Staphylococcus aureus* binds to filaggrins

Thomas P. Smith and Christopher J. Bailey*

Department of Biochemistry, Trinity College, Dublin 2, Ireland

Received 16 October 1985; revised version received 31 October 1985

The affinity of epidermolytic toxin from *Staphylococcus aureus* for proteins from the target tissue has been tested by a Western blotting procedure. Particular proteins in a 1 M phosphate extract of epidermis reacted on nitrocellulose blots with a probe prepared by the conjugation of toxin with peroxidase. Protein extracted into 50 mM Tris-HCl did not react. The probe detected profilaggrin, filaggrin and a smaller unidentified polypeptide. It is suggested that the interaction is relevant to the mode of action of the toxin.

1. INTRODUCTION

Epidermolytic toxins [1,2] from *Staphylococcus aureus* cause splitting of the skin in man and other susceptible species [3]. The lesion occurs at the level of the stratum granulosum without marked cell cytolysis [4] or any evidence of binding of the protein to the tissue [5]; the mode of action of the toxin has thus remained unknown. However, in histochemical studies (unpublished) we have obtained evidence that the toxin binds to keratohylin granules [6] in the epidermis. This encouraged us to develop a Western blotting procedure to search for an affinity between epidermolytic toxin and epidermal proteins. Here we show that toxin selectively binds to the filaggrin group [7,8] of proteins, some of which occur in keratohylin granules.

2. EXPERIMENTAL

2.1. Materials

Epidermolytic toxin type B was purified as described [2]. Glutaraldehyde was purified by the procedure of Anderson [9]. Horseradish peroxidase, *M*, standards and 3,3′-diaminobenzidine were obtained from Sigma.

2.2. Epidermal extracts

Whole thickness skin was obtained by dissection of neonatal mice (<2 days old). The epidermis was separated by immersion in phosphate-buffered saline, pH 7.4, containing 10 mM EDTA at 56°C for 30 s, then pulverized in liquid nitrogen. Extracts were prepared by homogenization in extraction buffer (1 ml/40 mg) and clarified by centrifugation.

2.3. Peroxidase-conjugated toxin preparation

Epidermolytic toxin was conjugated to peroxidase by the glutaraldehyde [10] procedure. The reaction mixture was purified by gel chromatography on Sephadex G-100. Assays for toxin and peroxidase activities showed that the peroxidase-conjugated toxin fraction was completely free from unreacted toxin and partially separated from unreacted peroxidase.

2.4. Polyacrylamide gel electrophoresis and blotting procedures

Protein samples were analysed by electrophoresis and blotted onto nitrocellulose as described in the Experimental section.

* To whom correspondence should be addressed
trophoresis in 12.5% gels [11]. One of a pair of duplicate sample tracks was taken for Coomassie blue staining. The other was blotted by electrophoresis onto a nitrocellulose membrane [12], which was then incubated overnight with toxin-peroxidase conjugate. The washed transfer was developed by incubation in 0.04% (w/v) diaminobenzidine, 0.03% (v/v) H₂O₂ in citrate-phosphate buffer, pH 5.0. Reaction was stopped by immersion in water.

2.5. Amino acid analysis
Proteins were freeze-dried from volatile buffer, hydrolysed in 6 M HCl at 110°C for 24 h and analysed by ion-exchange chromatography [13].

3. RESULTS AND DISCUSSION
Epidermal extracts in 50 mM Tris-HCl, pH 7, displayed a complex pattern on SDS gels stained with Coomassie blue (fig.2, track B). When the proteins were electrophoretically transferred to nitrocellulose they displayed negligible affinity towards the toxin probe even at high loadings (fig.2, track E). Similarly a set of standard proteins (fig.2, track C) was also unreactive (fig.2, track F). A keratohylin granule fraction was prepared by extraction of whole epidermis into 1 M potassium phosphate, pH 7, followed by dilution to 0.1 M and centrifugation. The pellet contains material from keratohylin granules [14]. Proteins in this fraction, after electrophoresis and transfer, reacted positively with the toxin probe. As shown in fig.1b, 3 groups of bands were detected. The slowest, often accompanied by an indistinct smear, corresponded to a polypeptide of 360 kDa, as judged by extrapolation (the largest standard protein was myosin, 205 kDa); a complex group had a molecular mass of ~30 kDa; the third positive band had a value of 16.5 kDa. When toxin-

![Fig.1. SDS-polyacrylamide gel electrophoresis of keratohylin granule extracts. The sample was prepared by precipitation at 0.1 M phosphate of whole epidermal tissue protein in 1 M potassium phosphate, pH 7.0 (a) stained with Coomassie blue, (b) transferred to nitrocellulose membrane and challenged with toxin-peroxidase conjugate. The arrowed positions mark the mobilities of a standard set of proteins shown in fig.2.](image1)

![Fig.2. SDS-polyacrylamide gel electrophoresis of epidermal preparations (a) stained with Coomassie blue, (b) transferred to nitrocellulose and challenged with toxin-peroxidase conjugate. Tracks: (A, D) filaggrin purified by column chromatography; (B, E) epidermal tissue extracted into 50 mM Tris-HCl; (C, F) standard proteins, bovine serum albumin, ovalbumin, glyceraldehyde-3-phosphate dehydrogenase, carbonic anhydrase, trypsinogen, trypsin inhibitor and a-lactalbumin.](image2)
peroxidase conjugate was omitted from the protocol or replaced by horseradish peroxidase, no colour development occurred with keratohylin granule samples.

The very large polypeptide in keratohylin granule extracts should be profilaggrin [8,15]. To confirm the identification, profilaggrin was purified from 1 M phosphate solution by selective precipitation at 0.4 M phosphate. The pure material had a mobility corresponding to 360 kDa, gave a positive result for phosphoprotein by the 'Stains-all' procedure [6], had the expected distinctive amino acid composition (table 1) and reacted with toxin-peroxidase conjugate on nitrocellulose transfers (fig.3).

The band(s) at 30 kDa have a mobility corresponding to filaggrin [17,18]. Accordingly, epidermal extracts in 1 M NaSCN were precipitated by dilution with water and separated by chromatography on Bio-Gel P-200 in 10% (w/v) formic acid adjusted to pH 2.0 [19]. The preparation was resolved into a doublet with electrophoretic mobility corresponding to 30 kDa (fig.2, track A), was not a phosphoprotein and reacted with the toxin-peroxidase conjugate (fig.2, track D). The composition of the material, notably the dominating amounts of His, Arg, Ser, Glx, Gly, Ala, is typical of filaggrin (table 1).

Table 1

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Profilaggrin (mol%)</th>
<th>Filaggrin (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This work [19]</td>
<td>This work [7]</td>
</tr>
<tr>
<td>Lys</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td>His</td>
<td>6.9</td>
<td>8.6</td>
</tr>
<tr>
<td>Arg</td>
<td>12.0</td>
<td>12.3</td>
</tr>
<tr>
<td>Asx</td>
<td>6.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Thr</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Ser</td>
<td>16.7</td>
<td>19.9</td>
</tr>
<tr>
<td>Glx</td>
<td>19.1</td>
<td>19.1</td>
</tr>
<tr>
<td>Pro</td>
<td>3.1</td>
<td>3.8</td>
</tr>
<tr>
<td>Gly</td>
<td>14.7</td>
<td>16.0</td>
</tr>
<tr>
<td>Ala</td>
<td>8.5</td>
<td>9.9</td>
</tr>
<tr>
<td>Cys</td>
<td>0.4</td>
<td>0 (&lt;0.4)</td>
</tr>
<tr>
<td>Val</td>
<td>3.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Met</td>
<td>0.3</td>
<td>0 (&lt;0.2)</td>
</tr>
<tr>
<td>lle</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Leu</td>
<td>2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Tyr</td>
<td>1.4</td>
<td>0 (&lt;0.2)</td>
</tr>
<tr>
<td>Phe</td>
<td>1.1</td>
<td>0 (&lt;0.2)</td>
</tr>
</tbody>
</table>

Fig.3. SDS-polyacrylamide gel electrophoresis of purified profilaggrin on 5% gels. (a) Stained with Coomassie blue; (b) transferred to nitrocellulose and reacted with toxin-peroxidase conjugate. Tracks (A, C) profilaggrin preparation; (B) standard proteins myosin, β-galactosidase and phosphorylase b. Mr, values of the standards are indicated by the arrows.

The identity of the third and smallest polypeptide observed on the blots is less obvious. It is known that profilaggrin breaks down to filaggrin [8] which is further depolymerized to amino acids [20]. It may be that the 16.5 kDa protein is a product of these catabolic events.

In view of the high degree of binding specificity between the toxin conjugate and the filaggrin group of proteins, it seems likely that this interaction is important for the biological activity of the toxin. It is not clear how binding of toxin to an intracellular granule protein could lead directly to loss of adhesion at the cell surface. However, an indirect connection does exist, for the keratohylin granules are linked to the keratin intermediate filament network which extends to the cell surface, notably to desmosomes [21].

311
This theory of the action of epidermolytic toxin has a number of other requisite properties. For example, the absence of epidermal cell cytolysis during toxicosis is explicable in terms of the specificity of intracellular action; the marked tissue specificity of the toxin may be seen to reflect the distribution of the tissue-specific target protein, filaggrin.

ACKNOWLEDGEMENTS

We thank Drs J.P. Arbuthnott and J. De Azavedo for useful discussions and the Medical Research Council of Ireland for a grant in support of this work.

REFERENCES