A circular-dichroism study of epidermolytic toxins A and B from *Staphylococcus aureus*

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The far-u.v. circular-dichroism spectra of the two epidermolytic toxins was analysed into fractional contributions of 0.09 helix and 0.46 β -sheet to each toxin structure. Trifluoroethanol perturbation caused an initial increase in dichroic absorption at 205 nm and then a change characterized as a β -sheet-to- α -helix transition. The intense near-u.v. spectra suggested that the toxins have unusually rigid, though different, aromatic-side-chain arrangements.

The epidermolytic toxins from *Staphylococcus aureus* are extracellular proteins that cause the splitting of human skin at the epidermal layer during infection of infants. Two antigenically distinct proteins of identical activity have been isolated by three groups of workers (Arbuthnott & Billcliffe, 1976; Kondo *et al.*, 1976; Wiley & Rogolsky, 1977). More recent evidence (Johnson *et al.*, 1979; Bailey *et al.*, 1980) suggests that each group has independently isolated the same two serotypes. With the agreement of other workers in the field we shall use the designations 'type A' (previously 'type i') and 'type B' (previously 'type ii'). Although the two toxins are encoded differently, by chromosomal (type A) or plasmid (type B) genes (Rogolsky *et al.*, 1976; O'Reilly *et al.*, 1979), are antigenically distinct and share few, if any, common tryptic peptides (Bailey *et al.*, 1980), *N*-terminal sequence determination (Johnson *et al.*, 1979) of the initial 20–26 residues supports the idea of a homologous relationship, as might be expected, since the activity appears to be unique to *S. aureus*.

In the present paper we report that the far-u.v. c.d. spectra indicate a high degree of conformational similarity and a similar response to the perturbant trifluoroethanol. Although the near-u.v. spectra are quite different from each other, they are both very intense, possibly indicating a very rigid conformation about the aromatic side chains.

Experimental

Toxin samples

The two proteins were purified as previously described (Bailey *et al.*, 1980), with the addition of a

chromatographic separation on Sephadex G-50 (superfine grade) in 0.01 M-NH₄HCO₃ as a final stage. The pure preparations were stored as freeze-dried powders.

Tryptophan determination

The fluorescence emission spectrum of the proteins was scanned with a Perkin–Elmer MPF-443 spectrofluorimeter. The content of tryptophan was assessed qualitatively or semiquantitatively by examining emission spectra in the range 300–360 nm. Tryptophan released by hydrolysis *in vacuo* in 3 M-toluenesulphonic acid (Liu & Chang, 1971) or 4 M-NaOH (Hugli & Moore, 1972) was measured after ion-exchange chromatography on a JEOL 5-AH amino acid analyser.

Circular dichroism

Toxin samples were studied in distilled water and in water diluted with the required volume of 2,2,2-trifluoroethanol (BDH; 'Special for Spectroscopy' grade). Spectra were recorded digitally from 340 to 250 nm and from 260 to 200 nm with a Jasco J41-C spectropolarimeter equipped with a J-DPY data processor, with sensitivity of 0.5 millidegrees/cm and with an instrumental time constant of 16 s. Fused silica cells of path length 1, 2 and 10 mm were used as required for near- and far-u.v. Reported spectra were recorded at 15°C and represent the average from at least two scans. Spectra are expressed as molar c.d. absorption ($\Delta\epsilon$), based on a mean residue weight of 110 for far-u.v. spectra and a molar mass of 30 000 or 29 500 (type A and B respectively) for the near-u.v. spectra.

Results and discussion

Specific absorption coefficients in the near-u.v., estimated by adsorption measurements on solutions of known amino acid residue content, were 8.4 ± 0.3 (2) at 277 nm for type A and 8.2 ± 0.2 (3) at 278 nm for type B. The spectra were independent of pH over the range 6.8–8.0. The value for type A is identical with that given by Johnson *et al.* (1975).

The near-u.v. c.d. spectra (Fig. 1) of the toxins are unusually intense. The tryptophan content of both proteins was therefore re-examined in order to test the possibility that the content of this amino acid had been underestimated. The fluorescent emission spectra of type B in water had a peak at 304 nm (uncorrected), which confirms the absence of tryptophan (Bailey *et al.*, 1980) in this protein. Type A toxin in water had a fluorescent emission peak at 336 nm, indicating the presence of tryptophan. The intensity of this spectrum, in 6 M-guanidinium chloride, was consistent with the presence of a single tryptophan residue. Chromatographic estimation after hydrolysis in toluenesulphonic acid gave 0.86 mol of tryptophan per mol of protein (M_r 30000). The recovery of tryptophan by this procedure, measured in parallel experiments with similar

amounts of protein, was 80% and 77% for hen's-egg lysozyme and bovine serum albumin respectively. Alkaline hydrolysis for 18 h gave 0.99 ± 0.04 (4) mol of tryptophan/mol of protein; after 93 h hydrolysis the corresponding value was 1.02 ± 0.05 (2). These hydrolytic values confirm that toxin A contains a single tryptophan residue.

The near-u.v. c.d. spectrum of toxin A has a dominant negative feature at 275 nm and a positive peak at 291 nm. The latter peak (Fig. 1, insert) is at a longer wavelength than that generally observed for tyrosine (Horwitz *et al.*, 1970; Strickland *et al.*, 1969) and must be due to tryptophan. However, the peak has an extraordinary intensity ($\Delta\epsilon = 3.5 \text{ M}^{-1} \cdot \text{cm}^{-1}$) for a single tryptophan residue. The most intense spectrum of a model compound is that of *N*-acetyltryptophanamide at 77 K; it has a $\Delta\epsilon$ of about $1.4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Strickland *et al.*, 1969). However, in the proteins azurin and apoazurin, containing a single tightly packed (Grinvald *et al.*, 1975) tryptophan, the value approaches $+2 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at 292 nm (Strickland, 1974). In seeking explanations for the unusual near-u.v. spectral properties observed in toxin A, immobilization by incorporation into a highly specific and rigid conformation appears the most reasonable mechan-

Fig. 1. Near-u.v. c.d. absorption spectra of toxin A (●) and toxin B (■) in water. The insert shows a multi-scan spectrum of toxin A having no significant absorption above 300 nm.

Fig. 2. Far-u.v. c.d. absorption spectra of epidermolytic toxins
(a) Toxin A in 0 (●), 25 (▲) and 42% (■) (v/v) trifluoroethanol; (b) Toxin B in 0 (●), 20 (▲) and 53% (■) trifluoroethanol.

ism to give enhanced c.d. intensity. It may be noted that the conformation of toxin A is outstandingly stable, with the activity being fully retained after more than 20 min at 100°C (Kapral & Miller, 1971; Kondo *et al.*, 1973).

The type B near-u.v. spectrum has a main peak at 276 nm, a smaller one at 288.8 nm, and a strong shoulder at 282 nm. Again the spectra have extraordinary intensities ($\Delta\epsilon_{\text{max.}} \sim 10$) requiring additive contributions (Strickland, 1974) from a large number of the sixteen total tyrosine residues (assumed $\Delta\epsilon_{\text{max.}} \sim 1$ each). Other than the strength of the observed transitions, there is no evidence of a relation between the toxin spectra and therefore no evidence for identical environments of the tyrosine side chains of the two proteins. Similar arguments concerning immobilization may be relevant in this case also.

The far-u.v. spectra (Fig. 2) were analysed into fractional contributions of about 0.09 helix and 0.45 β -sheet to each protein conformation (Table 1). When the structures were perturbed with trifluoro-

Table 1. Multicomponent analysis of far-u.v. c.d. of epidermolytic toxin spectra by the CONTIN procedure (Provencher & Glockner, 1981)

The analyses were of 160-point averaged data in the range 240 nm–200.25 nm and the single precision procedure was usually used with weighting against data from below 210 nm. From these unsmoothed data (Fig. 2) the CONTIN analysis generated smooth curves, indistinguishable from the visual fits except in three cases (type B in 0, 10 and 36% trifluoroethanol), where the predicted curve fitted residual noise at 200–205 nm. For these three cases the analyses of data visually smoothed below 205 nm only are presented. f_H , f_β , f_T and f_R are the computed fractional contents of helical, β -sheet, β -turn and remainder structural components respectively.

Protein	[Trifluoroethanol] (%, v/v)				
		f_H	f_β	f_T	f_R
Toxin A	0	0.08	0.46	0.15	0.32
	16.6	0.16	0.44	0.13	0.27
	20.8	0.15	0.40	0.08	0.37
	25.0	0.17	0.33	0.09	0.42
	29.1	0.15	0.42	0.11	0.31
	33.3	0.21	0.49	0.04	0.26
	41.6	0.27	0.33	0.05	0.34
	75.0	0.63	0.00	0.00	0.37
Toxin B	0	0.09	0.45	0.14	0.33
	10.0	0.12	0.52	0.00	0.36
	20.0	0.12	0.45	0.08	0.34
	30.0	0.08	0.36	0.14	0.41
	36.6	0.12	0.46	0.11	0.31
	53.3	0.36	0.29	0.06	0.28
	75.0	0.51	0.13	0.00	0.36

Fig. 3. Molar c.d. absorption coefficients at 205 nm of epidermolytic toxins A (●) and B (□) as a function of trifluoroethanol concentration

ethanol, two spectral transitions were observed. At high concentrations of perturbant the expected promotion of α -helix was observed (Fig. 2) and CONTIN analysis (Provencher & Glockner, 1981) (Table 1) suggested that a β -sheet-to- α -helix transition was taking place. However, at lower concentrations of trifluoroethanol, a unique transition characterized by a high intensity at 205 nm was observed. Plotting the data as in Fig. 3 indicated that the two effects can be separated. CONTIN analysis of spectra at 10–30% (v/v) trifluoroethanol suggested that a small increase in helix content of toxin A, and possibly of toxin B, had occurred. β -Sheet assessment by the Provencher & Glockner (1981) procedure is less accurate, and the small transient decreases in β -sheet content that occur over the range 0–35% trifluoroethanol may not be significant. It seems likely that this initial transition involves the formation of more helix, and some complementary change, as yet uncertain, in a small part of the structure.

The identical contents of helix and β -sheet, and the unique response to trifluoroethanol, support the proposal of a homologous relation between the toxins (Johnson *et al.*, 1979). The comparative studies reported here suggest that a broadly similar chain-folding is present in which aromatic side chains are embedded, possibly in a more rigid matrix than is usual for globular proteins, thus accounting for the intense near-u.v. effects observed.

It is noteworthy that the view that epidermolytic toxin acts without binding to cells (Baker *et al.*, 1978) has recently been contradicted by new evidence from an assay procedure *in vitro* (Nishioka *et al.*, 1981) that has indicated indirectly that lysosomes bind toxins. The rigid conformations detected in the present study should make the toxins proteinase-resistant, a necessary property if a digestive organelle is the target site.

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