THE AMINO ACID SEQUENCE OF THE DELTA HAEMOLYSIN OF *STAPHYLOCOCCUS AUREUS*

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Received 17 April 1980

1. Introduction

The δ haemolysin of *Staphylococcus aureus* is one of a number of extracellular cytolytic and cytotoxic polypeptides produced by most strains of the micro-organism [1–4] and is notable for its physico–chemical and biological properties. It is stable to boiling and soluble in chloroform–methanol (2:1, v/v) and aqueous solutions (reviewed [4]) and is inhibited by phospholipids, fatty acids, and normal serum lipoproteins [5–7]. Three different purification procedures [8–10] yield material with broadly similar properties and an amino acid analysis which is notable for the absence of arginine, proline, histidine, tyrosine and cysteine, and the presence of a high proportion of hydrophobic amino acids. Although most earlier studies suggested that native δ haemolysin was likely to have mol. wt >100 000, it was apparent from gel permeation chromatography under denaturing conditions that the minimum molecular weight for the polypeptide subunit was likely to be <6000 [10]. This investigation of the primary structure of δ haemolysin was prompted by the realization that the solvent-transfer method [8] provided substantial quantities of highly purified δ haemolysin from culture filtrates of *S. aureus*, that it did so more rapidly and reproducibly than other methods [7,8] and that the polypeptide shared all of the characteristic amphipathic and cytolytic properties observed with preparations of δ haemolysin previously purified by ion exchange or adsorption chromatography and by gel filtration.

The most striking properties of δ haemolysin are its 'surface-active' effects on the membranes of a wide variety of cells and organelles and its functional similarity to the effects of bee venom melittin (reviewed [4]). We have observed that δ haemolysin also shares important structural features with melittin and wish to call attention to possible implications arising from the structure of δ haemolysin which may be pertinent to current views of the structure and assembly of membranes and the processing of secreted proteins.

2. Materials and methods

Preliminary studies on the structure of δ haemolysin were carried out on material purified by the solvent-transfer method from culture supernatants of *S. aureus* strain 186X [8], kindly supplied by N. G. Heatley. Subsequent experiments were performed with purified δ haemolysin purified by the same procedure but from *S. aureus* strain RN25, provided by R. Novick. Strain RN25 is a derivative of strain RN1. The latter is lysogenic for at least 3 staphylococcal phages (φ11, φ12, and φ13) whereas strain RN25 appears to harbor only phage φ13 (R. Novick, personal communication). Delta haemolysin was assayed as in [8]. When silica gel thin-layer chromatography indicated the presence of ninhydrin-positive minor impurities in the preparations of δ haemolysin purified by the solvent-transfer method, the material was further purified by chromatography on *n*-octyl-Sepharose (Pharmacia, London) as in [11].

The amino acid composition of δ haemolysin was determined by extrapolation in the usual fashion after hydrolysis of the pure polypeptide at 105°C for 24, 48 and 72 h in 6 N HCl and in evacuated tubes. Peptides arising from enzymic digests were hydrolysed

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for 27 h. Tryptophan was determined by hydrolysis with 3 N mercaptoethane sulfonic acid at 105°C for 24 h. All analyses were performed on a standard single column amino acid analyser (Locarte Ltd, London) operating with sodium citrate buffers.

Tryptic digestion of δ haemolysin (10 mg/ml) was performed at 37°C in 50 mM ammonium bicarbonate (pH 8.0) with bovine trypsin (0.1 mg/ml) recystallised in the presence of diphenylcarbamylchloride (BDH, Poole). The digestion was terminated after 4 h by freezing and lyophilisation and the peptides were purified by high-voltage paper electrophoresis in volatile buffers at pH 6.5 and pH 2.1. Peptides were located by reaction with dilute fluorescamine and were eluted with 5% (v/v) ammonia prior to lyophilisation and sequence analysis by the dansyl-Edman procedure [12]. The C-terminal sequence of δ haemolysin was determined with a mixture of carboxypeptidases A and B (Sigma, Poole) under similar conditions to those described for trypsin. Samples were taken at 10 min intervals, lyophilised, and analyzed directly for the release of free amino acids Delta haemolysin and the larger C-terminal lysine tryptic peptides were coupled to amino propyl glass (7.5 nm porosity) by the diisothiocyanate method using 0.4 M dimethylallylamine coupling buffer (pH 9.5) as in [13].

3. Results and discussion

The amino acid composition of δ haemolysin is given in table 1 and compared with that expected for the determined amino acid sequence. The agreement with [8–10] is good but not sufficiently to suggest that all preparations studied were of comparable purity or that the δ haemolysin from each strain of *S. aureus* has the same primary structure.

Table 2 lists the tryptic peptides isolated by high-voltage paper electrophoresis and indicates the % final yield of each as well as the amino acid composition and the structure deduced by dansyl-Edman degradation. Comparison of the peptides with the sequence in table 3 suggests that anomolous cleavage occurred after Ile_9, Trp_{15} and Phe_{23}. The failure of peptides TE and TF to sequence well suggested that they both might be blocked. When both

Table 1
Amino acid analysis of δ haemolysin[c]

Amino acid	mol %	mol residue/ mol haemolysin[a]	Residues found in sequence
Aspartic acid	16.0	4.2	4
Threonine	11.4	3.0	3
Serine	4.6	1.2	1
Glutamic acid	4.2	1.1	1
Proline	–	0.0	0
Glycine	4.6	1.2	1
Alanine	4.2	1.1	1
Cysteine[b]	–	0.0	0
Valine	8.8	2.3	2
Methionine	3.0	0.8	1
Isoleucine	17.1	4.5	5
Leucine	4.2	1.1	1
Tyrosine	–	0.0	0
Phenylalanine	3.8	1.0	1
Histidine	–	0.0	0
Lysine	15.6	4.1	4
Tryptophan	2.7	0.7	1
Arginine	–	0.0	0
Total residues		26.3	26

[a] Best near-integral values obtained after 24, 48 and 72 h hydrolysis and adjustment to fit a 26 residue amino acid sequence (mol. wt 2977)
[b] Analysis for cysteic acid after performic acid oxidation
[c] Analysis on δ haemolysin isolated by the solvent-transfer method [8] and purified further by chromatography on octyl-Sepharose [11]

Table 2
Tryptic peptides of delta haemolysin[b]

Peptide[a]	Composition	Yield (percent)	Sequence (and position)[c]	
TA	Lys	20	Lys	(26)
TB	Thr(0.9); Lys(2.0)	11	Thr-Lys-Lys	(24-26)
TC	Thr(1.0); Phe(0.7); Lys(1.7)	18	Phe-Thr-Lys-Lys	(23-26)
TD	Thr(1.1); Phe(0.8); Lys(1.0)	20	Phe-Thr-Lys	(23-25)
TE	Asx(3.8); Thr(1.7); Ser(1.1); Glx(1.1); Gly(0.9); Ala(1.0); Val(1.3); Met(0.8); Ile(2.0); Leu(0.6); Lys(0.6)	8	*Met-Ala-Glx-( )-Val-Asn-Lys	(1-22)
TF	Asx(2.2); Thr(0.9); Ser(0.9); Glx(1.0); Gly(0.9); Ala(0.9); Val(0.8); Met(0.8); Ile(1.6); Leu(0.9); Lys(0.6)	35	*Met-Ala-Gln-Asp-Ile-Ile-Ser···	(1-14)
TNB	Asx(1.0); Gly(1.0); Val(0.7); Leu(1.0); Lys(0.7)	10	Gly-Asp-Leu-Val-Lys	(10-14)
TNC	Asx(2.4); Thr(1.0); Val(0.9); Ile(0.6); Lys(1.0)	7	Ile-Ile-Asp-Thr-Val-Asn-Lys	(16-22)
TNE	(a) Phe (Free amino acids)	7	Phe	(23)
	(b) Trp	13	Trp	(15)

[a] Arbitrary designations; at pH 6.5 peptides TA through TD were (+); TE and TF were (−) and remainder were neutral
[b] 1 μmol δ haemolysin (3 mg) digested as in section 2
[c] Positions verified by N-terminal sequence of δ haemolysin and carboxypeptidase digestion. Peptides TE and TF were blocked and gave sequence after treatment with methanolic HCl (see text)

of these peptides and delta haemolysin were treated with 1 M HCl in methanol at room temperature for 2 h, the material remaining after lyophilisation gave strong PTH signals at each round of the Edman degradation on solid phase (22 residues of δ haemolysin and 7 for peptide TF) and the first 3 residues of TE were identified by the manual dansyl-Edman procedure. The 3 terminal residues of peptide TE were determined by carboxypeptidase as were residues 23–26 of the intact haemolysin.

The blocked N-terminus of δ haemolysin and peptide TF was judged to be *N*-formyl methionine by mass spectrometry of both polypeptides after deuteroacetylation and permethylation [14]. Both samples yielded signals corresponding to f-Met (*m/e* 174) and f-Met–Ala (*m/e* 259). We conclude therefore that the structure given in table 3 is the complete amino acid sequence of the δ haemolysin purified from culture supernatants of *S. aureus* strain 186X as in [8]. As isolated, ~10% of the material exists as unformylated amino terminal methionine. Experiments are in progress to compare the cytolytic specific activities of the two species. It should be noted that the apparent isoelectric point of δ haemolysin in the presence of a non-ionic detergent (0.1% Tween 80) has been reported to be pH 5.5 [10]. This observation is compatible with the proposed structure for δ haemolysin with f-Met at the amino terminus but seems less likely for the same polypeptide with a free (unblocked) N-terminal methionine.

When the proposed primary structure of δ haemolysin is compared with that of melittin from *Apis molifera* venom [15] and the broadly similar properties of both molecules are considered, it is apparent that their membrane-specific and physical properties are likely to have a common structural basis. Experiments are in progress to compare further the unusual physico–chemical properties of δ haemolysin [16] with those of melittin, for which there are now useful data [17–20]. We also call attention to an important insight which may be gained from the knowledge that δ haemolysin is an extra cellular amphipathic peptide with a central 'core' of hydrophobic residues, is 26 amino acids in length and has *N*-formyl methionine at the amino terminus. We suggest that δ haemolysin is an ideal candidate for the 'signal sequence' of one or more secreted or membrane-associated proteins [22–24]. The affinity of δ haemolysin for phospholipids which underlies its cytolytic properties may thus be seen to be a direct consequence of a primary role in effecting or facilitating the extrusion or insertion of a larger polypeptide through or into the cytoplasmic membrane.

Table 3
Primary structure of δ haemolysin (DHL)[a] and comparison with bee venom melittin (BVM)[b]

				1				5				10				15				20				25	
DHL[c]	H_3N-	Met-	Ala-	Gln-	Asp-	Ile-	Ile-	Ser-	Thr-	Ile-	Gly-	Asp-	Leu-	Val-	Lys-	Trp-	Ile-	Ile-	Asp-	Thr-	Val-	Asn-	Lys-	Phe-	Thr-	Lys-	Lys-	COO
				(+)				(-)						(-)		(+)			(-)			(+)			(+)	(+)	(-)
				(+)				(+)												(+)	(+)	(+)	(+)				
BVM	H_3N-	Gly-	Ile-	Gly-	Ala-	Val-	Leu-	Lys-	Val-	Leu-	Thr-	Thr-	Gly-	Leu-	Pro-	Ala-	Leu-	Ile-	Ser-	Trp-	Ile-	Lys-	Arg-	Lys-	Arg-	Gln-	Gln-	$CONH_2$
				1				5				10				15				20				25				

[a] Sequence shown with free α-amino group at N-terminus; isolated as *N*-formyl Met; see text. Formal charge indicated for groups likely to be ionised over pH 6–8;
[b] Melittin of *Apis molifera* [15] – other variants are known
[c] Additional variants have been studied using purified δ haemolysin from other strains of *S. aureus*. A canine strain [25] gave Val_{10}, Glu_{11}, Ala_{17} and Glu_{18} (J. E. F., W. H. Turner, W. V. S. unpublished)

Acknowledgements

The authors are grateful to Norman Heatley for his generous supply of purified δ haemolysin and for his interest and encouragement. This study was supported by the Medical Research Council and by the Science Research Council. J. E. F. holds a research training fellowship of the MRC. We thank John Walker of the Laboratory of Molecular Biology, Cambridge, for N-terminal sequence data on unblocked δ haemolysin using the Beckman Sequenator. We are grateful to Gillian Smith for amino acid sequence data on δ haemolysin purified by other methods and communicated in preliminary form at the Dublin meeting (Sept. 1979) of the Society for General Microbiology [26].

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