PHYSICOCHEMICAL STUDIES ON DELTA HAEMOLYSIN, A STAPHYLOCOCCAL CYTOLYTIC POLYPEPTIDE

John E. FITTON

Department of Biochemistry, University of Sheffield, Sheffield S10 2TN, England

1. Introduction

Delta haemolysin is one of a number of extracellular cytolytic polypeptides produced by many isolates of *Staphylococcus aureus* [1]. It is heat stable, soluble in both organic solvents (e.g., chloroform: methanol, 2:1, v/v) and aqueous solutions and is inhibited by phospholipids, fatty acids and serum lipoproteins. The toxin is surface active [2] and exhibits pronounced effects on the membranes of a wide variety of cells and organelles. Recent results suggest that delta haemolysin is a potential tumor promoter at sublytic concentrations [3].

M_r estimates have ranged from 5200 to 195 000 and it has been suggested that the toxin exists as a multimeric assembly of identical subunits [4]. The toxin monomer has been shown to consist of 26 amino acid residues, contains no proline, cysteine, tyrosine, histidine or arginine and has an N-terminal formyl-methionine residue [5]. The cytolytic effects of delta toxin and melittin, the 26 amino acid residue surface active toxin isolated from bee venom are similar although distinguishable from the cytolytic effects of Triton X-100 [6,7].

The studies described here were designed to investigate and clarify the conflicting relative molecular mass estimates and to provide information about the conformation of the toxin in free solution and in 'pseudo membrane' environments using the techniques of fluorescence spectroscopy and gel filtration enabling a more detailed conformational comparison between delta haemolysin and the extensively studied toxin, melittin to be made.

2. Materials and methods

Sephadex and Sepharose gel filtration media were obtained from Pharmacia (GB) Limited, London, phosphatidylcholine was from Sigma Chemical Company Limited, Poole, Dorset, England, Brij 56 was from Honeywell-Atlas Limited, Carshalton, Surrey, England; all other chemicals were of the highest quality commercially available.

Delta haemolysin was purified from culture filtrates of *S. aureus* strain 186X [8] as previously described [5] using a combination of the solvent-transfer method [8] and chromatography on n-octyl-Sepharose [9]. Partially purified toxin was kindly provided by Dr N. Heatley.

Mixed micelles of delta haemolysin—phosphatidylcholine were prepared by dissolving the toxin and phosphatidylcholine in chloroform:methanol (2:1, v/v) followed by evaporation to give a thin film. This was taken up in the required solvent by mechanical shaking and finally sonicated for five minutes.

M_r estimates of delta haemolysin in a variety of solvents were determined by gel filtration (1 × 50 cm column) using Sephadex G50 or Sepharose 6B as required. Aliquots (0.25–0.5 mg) of toxin were loaded onto the column and the eluant monitored by absorbance at 280 nm.

Fluorescence measurements were performed at 20°C on a Perkin-Elmer 3000 fluorimeter. The excitation wavelength was 280 nm. Unless otherwise stated, 1 ml samples of a 1.6×10^{-5} M toxin solution were used.

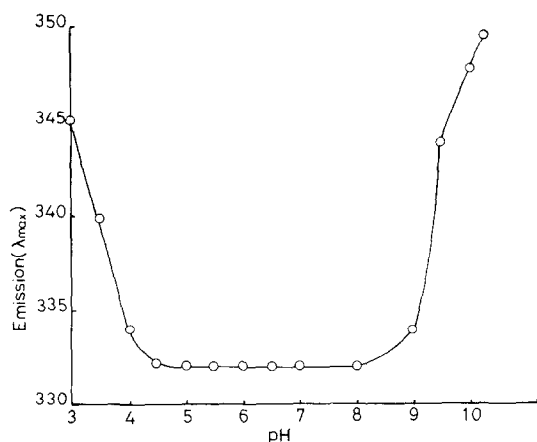## 3. Results

The fluorescence of the single-tryptophan residue

Fig.1. Variation of fluorescence emission maximum of delta toxin with change in pH. The buffer was 25 mM sodium acetate adjusted to the required pH with acetic acid or NaOH as required.

Fig.3. Variation of fluorescence emission maximum of delta toxin with increasing phosphatidylcholine (●) and detergent (□, Brij 56) concentration. Toxin-detergent micelles were prepared by addition of 1% w/v Brij 56 stock solution to toxin solutions. Solutions contained 20 mM sodium phosphate, pH 6.0.

at position 15 in the delta haemolysin amino acid sequence enables the conformation of the toxin to be monitored with great sensitivity in a variety of solvents. In an aqueous environment, at neutral pH, the toxin fluoresces at 332 nm when excited at 280 nm (fig.1). Below pH 4 and above pH 9 however, the emission maximum is redshifted to higher wavelengths, indicative of the indole ring of the tryptophan residue being transferred from a relatively apolar environment to a more polar environment. A similar redshift is observed with increasing guanidine–HCl concentration (data not shown), a marked transition occurring at 2 M concentration. Increasing alcohol concentrations elicit a similar redshift (fig.2), both
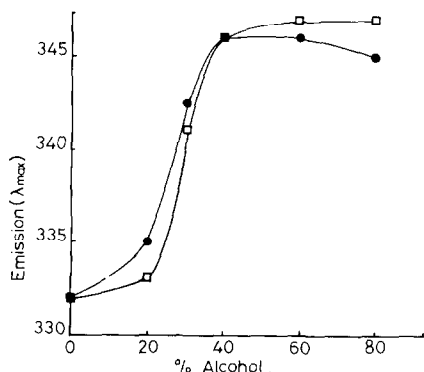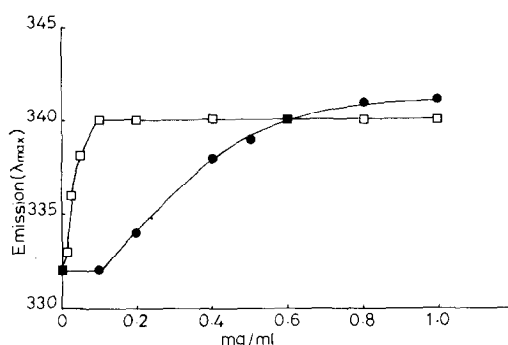
methanol and ethanol yielding a transition at an alcohol concentration of approx. 30%. In all cases an increase in emission wavelength is accompanied by an increase in the relative fluorescence intensity (data not shown).

The interaction of delta toxin with detergent micelles (non-ionic detergent Brij 56) and phosphatidylcholine liposomes was investigated by fluorescence spectroscopy. In both cases a marked redshift in emission maxima occurred with increasing detergent or phosphatidylcholine concentration (fig.3), concomitant with an increase in relative fluorescence intensity.
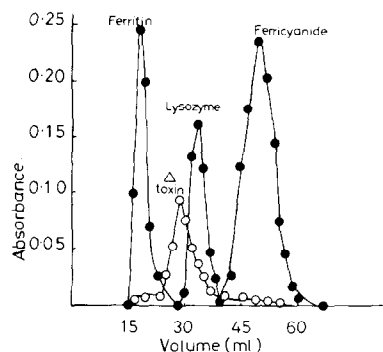
Gel filtration of delta toxin on Sephadex G-50 in

Fig.2. Variation of fluorescence emission maximum of delta toxin with increasing alcohol concentration. (□) Methanol, (●) ethanol.

Fig.4. Gel filtration of delta toxin on Sephadex G-50 (1 × 50 cm column). Eluting solution was 0.5% v/v acetic acid. Fractions were monitored at 280 nm for delta toxin and lysozyme and 400 nm for ferritin and potassium ferricyanide.

water or in 25 mM sodium phosphate buffer (pH 6.0) resulted in elution of the toxin in the void volume suggesting a high apparent molecular weight. Gel filtration on a calibrated Sepharose 6B column in the same buffer resulted in elution of the toxin ($K_d = 0.53$) just behind the elution position of catalase ($K_d = 0.45$) indicating an apparent M_r of 210 000, in reasonable agreement with the value of 195 000 determined by Kantor et al. [4].

Gel filtration in a calibrated Sephadex G-50 column in 0.5% acetic acid (fig.4), yields an apparent M_r 14 000 ($K_d = 0.23$), the toxin eluting just prior to lysozyme ($K_d = 0.32$). Similar elution profiles were obtained for the toxin when the eluting buffer was 50 mM NaOH, 70% methanol, or 6 M guanidine–HCl/ 25 mM sodium phosphate (pH 6) (data not shown) again suggesting an apparent M_r of 14 000.

4. Discussion

Although the delta toxin monomer consists of a 26 amino acid residue polypeptide [5], the gel filtration results reported in the present study show that the toxin is present as a tetramer in aqueous solution at extremes of pH and in aqueous alcoholic solution. The observation that the toxin is also tetrameric in buffered 6 M guanidine–HCl indicates that this form of the toxin is extremely stable in aqueous solution, indeed, monomeric toxin has not been observed under any aqueous solvent conditions employed to date, although monomers may be present in solvents of lower dielectric constant.

At neutral pH in aqueous solution the toxin has been shown to exist as a multimeric aggregate of M_r 210 000 consisting of 16–18 tetramers (64–72 monomers). The dissociation of the multimeric form of the toxin to tetramers at extremes of pH would indicate electrostatic bonding is of importance in the stabilisation of the multimeric aggregate.

The use of secondary structure analysis by the Chou and Fasman method [10,11] predicts that the toxin possesses two helical domains, residues 1–6 ($\langle P\alpha \rangle = 1.19$) and residues 11–18 ($\langle P\alpha \rangle = 1.09$) joined by a flexible hinge region (residues 7–10). The results of circular dichroism studies (Bayley and Fitton, unpublished results) support this view, the toxin being predominantly α-helical (80%) in aqueous solution (>50% ethanol) and somewhat less helical (40%) in aqueous solution at neutral pH.

Molecular models based on the known sequence of

delta toxin, incorporating the secondary structure conformation indicated by the use of structural predictive methods, possess clear regions of hydrophobic and hydrophilic character on opposing faces of the α-helix, in keeping with the amphipathic properties of the toxin and bears a remarkable resemblance to the proposed structure of the surface active toxin of bee venom, melittin [12].

The results derived from the study of the intrinsic fluorescence of the Trp 15 residue in the toxin would indicate that Trp 15 is in a hydrophobic environment at pH 6 in aqueous solution, consistent with the view that the toxin exists as a multimeric aggregate under these conditions, the tryptophan residue being buried within the multimeric structure of the toxin. At extremes of pH the toxin dissociates into tetramers so exposing the tryptophan residues to the environment with a consequent redshift in the emission maximum from 332 nm to 345–350 nm.

Although no evidence can be found for the existence of monomers in solution, due to the proposed amphipathic structure of the toxin, it is likely that this is the active form of the toxin following interaction with membranes. Indeed the observed fluorescence maximum in liposomes and detergent micelles (340–342 nm) is significantly below the emission maximum of the tetrameric form (345–350 nm) although higher than that found for multimeric toxin (332 nm) perhaps indicating the interface position of the tryptophan residue after binding to liposomes or detergent micelles.

Although no homologies exist between delta toxin and melittin at the primary structure level the results of this investigation are consistent with homologies at the secondary and tertiary structure levels and this is in agreement with the similarities in the cytolytic effects of the two toxins [6,7].

Melittin is known to exist as monomers or tetramers in aqueous solution [13,14] dependent on pH and ionic strength, in contrast delta toxin has been shown to exist as tetramers or oligomers. Although the two toxins appear to have similar common structural features and exert comparable cytolytic effects further consideration of the interaction of delta toxin with biological membranes must await a more detailed spectroscopic analysis.

Acknowledgements

References

[1] Rogolsky, M. (1979) Microbiol. Rev. 43, 320–360.

[2] Colacicco, G., Basu, M. K., Buckelew, A. R. and Bernheimer, A. W. (1977) Biochim. Biophys. Acta 465, 378–390.

[3] Umezawa, K., Weinstein, I. B. and Shaw, W. V. (1980) Biochim. Biophys. Res. Commun. 94 (2), 625–629.

[4] Kantor, J. S., Temples, B. and Shaw, W. V. (1972) Arch. Biochem. Biophys. 151, 142–156.

[5] Fitton, J. E., Dell, A. and Shaw, W. V. (1980) FEBS Lett. 115 (2), 209–212.

[6] Thelestam, M. and Mölley, R. (1975) Infect. Immun. 11, 640 648.

[7] Thelestam, M. and Mölley, R. (1975) Infect. Immun. 12, 225–232.

[8] Heatley, N. G. (1971) J. Gen. Microbiol. 69, 269–278.

[9] Nolte, F. S. and Kapral, F. A. (1979) Am. Soc. Microbiol. Annu. Meet. Abstr. p17.

[10] Fasman, G. D. and Chou, P. Y. (1974) Biochemistry 13, 211–244.

[11] Fasman, G. D., Chou, P. Y. and Adler, A. J. (1976) Biophys. J. 16, 1201–1238.

[12] Dawson, C. R., Drake, A. F., Helliwell, J. and Hider, R. C. (1978) Biochim. Biophys. Acta 510, 75–86.

[13] Habermann, E. (1972) Science 177, 314–322.

[14] Gaudie, J., Hanson, J. M., Rumyanek, F. D., Shipolini, R. A. and Vernon, C. A. (1976) Eur. J. Biochem. 61, 369–376.