Purification of RNA-core Induced Streptolysin S, and Isolation and Haemolytic Characteristics of the Carrier-free Toxin

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(Received 2 September 1985)

RNA-core (RNAase-resistant fraction of yeast RNA) induced streptolysin S (SLS) was purified (40% recovery) to apparent electrophoretic homogeneity by hydroxylapatite chromatography followed by gel filtration on Sephadex G-100 in the presence of 6 M-guanidine. HCl. The specific activity of the purified toxin was 3×10^6 haemolytic units (mg protein)⁻¹. The M_r of the toxin was below 4000 on the basis of SDS-PAGE and 20000 by gel filtration in guanidine. HCl. High-voltage isoelectric focusing of the purified toxin allowed the isolation of the carrier-free SLS peptide for the first time. This peptide was basic (pI 9.2) as compared to native SLS (pI 3.6). The native toxin and the peptide had similar haemolytic properties except for the high lability of the peptide, which was stabilized by RNA-core. The M_r of the denatured peptide was about 1800, as estimated by gel filtration.

INTRODUCTION

Two haemolytic and cytolytic toxins, streptolysin O (SLO) and streptolysin S (SLS), differentiated by Herbert & Todd (1944) are produced by group A streptococci (for reviews see Ginsburg, 1970; Alouf, 1980). SLO is a 60 kDa immunogenic protein released in conventional culture media throughout the exponential phase of growth (Dassy & Alouf, 1983). In contrast, SLS is a less well understood, non-immunogenic toxin which is never detected unless a carrier (also called inducer) is added to the culture or to resting cell suspensions (Bernheimer & Rodbart, 1948). Many chemically unrelated inducers have been reported, the most potent being RNAase-resistant fraction of yeast RNA (RNA-core) and lipoteichoic acids (see Alouf, 1980; Theodore & Calandra, 1981). The haemolytic activity can be transferred from one inducer to another (Ginsburg & Harris, 1963; Taketo & Taketo, 1964; Koyama, 1964; Calandra & Oginsky, 1975; Theodore & Calandra, 1981). This led to the concept that SLS, as first suggested by Bernheimer (1954), is a complex formed by a peptide associated with an inducer, the inducer acting as a carrier or stabilizer. The peptide was estimated to consist of 28 to 32 amino acids (Bernheimer, 1967; Lai *et al.*, 1978). Attempts to isolate a carrier-free haemolytic moiety never succeeded, suggesting that this moiety undergoes decay or denaturation when the complex is treated in ways designed to remove or destroy only the carrier (see Bernheimer, 1972).

The purification, to various extents, of RNA-core induced SLS has been reported (Koyama & Egami, 1963; Duncan & Mason, 1976; Hryniewicz *et al.*, 1978; Lai *et al.*, 1978), but no biochemical or immunochemical indications of the homogeneity of the purified preparations were provided, due to the lack of sensitive staining methods and immune sera.

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Abbreviations: BAEE, *N*-benzoylarginine ethyl ester; BHF, basic haemolytic fraction; HU, haemolytic unit; IEF, isoelectric focusing; RNA-core, RNAase-resistant fraction of yeast RNA; SLO, streptolysin O; SLS, streptolysin S; SRBC, sheep red blood cells; TPCK, *L*-1-tosylamide 2-phenylethyl chloromethyl ketone.

In addition to erythrocytes, SLS damages and lyses other eukaryotic cells, bacterial protoplasts (see Alouf, 1980) and liposomes composed of various phospholipids (Duncan & Buckingham, 1981). The lytic effect is inhibited by a variety of phospholipids (Elias *et al.*, 1966), suggesting that these components are involved in the cytolytic action of SLS.

We report in this article the purification to apparent homogeneity of RNA-core induced SLS and the isolation of a haemolytically active carrier-free peptide whose properties are investigated and compared with those of the purified RNA-core-SLS complex.

METHODS

Chemicals and biochemicals. Hydroxylapatite (Bio-gel HTP) was purchased from Bio-Rad. Trypan Blue, gramicidin D, aprotinin, bovine chain B insulin and RNA-core were from Sigma. Staphylococcal delta-toxin was kindly provided by Dr Monica Thelestam (Karolinska Institute, Stockholm, Sweden). Carboxypeptidase A (EC 3.4.17.1) (35 U mg⁻¹), carboxypeptidase B (EC 3.4.17.2) (70 U mg⁻¹) and α -chymotrypsin (EC 3.4.21.1) (45 U mg⁻¹) were from Worthington. TPCK-treated trypsin (EC 3.4.21.4, type XIII) (10⁴ BAEE U mg⁻¹) was from Sigma. Leucine aminopeptidase (EC 3.4.11.1) (100 U mg⁻¹), proteinase K (EC 3.4.21.14) (20 mU Anson mg⁻¹) and pronase E (type XIV) (7 U mg⁻¹) were from Merck.

Strain and growth conditions. *Streptococcus pyogenes* strain C203S group A was kindly supplied by Professor Alan W. Bernheimer, New York University School of Medicine. The lyophilized strain was grown at 37 °C without shaking in brain heart infusion broth (BHI, Difco) supplemented with 1% (w/v) maltose and 2% (w/v) sodium bicarbonate (BHI-BM).

Induction of streptolysin S. A 50 ml volume of an overnight culture was inoculated in 2.5 l BHI-BM and grown for 5 h at 37 °C. The culture was centrifuged at 12000 g for 25 min at 4 °C and the cell pellet washed in 100 mM-potassium phosphate buffer, pH 7.0, before resuspension to a final volume of 40 ml in induction phosphate buffer (IB) (100 mM-KH₂PO₄, 2 mM-MgSO₄; adjusted to pH 7.0 with NaOH) supplemented with 30 mM-maltose. The cell suspension (about 17 mg dry wt cocci ml⁻¹) was incubated for 5 min at 37 °C and then induced by adding 0.5 mg RNA-core per ml of suspension (2.9%, w/w, cocci) for 5 min without shaking. The cell suspension was then immediately centrifuged at 4 °C at 15000 g for 20 min. The supernate (crude SLS) was collected and supplemented with ammonium acetate (100 mM final concentration) to stabilize SLS as suggested by Lai *et al.* (1978). The pellet was then resuspended in 40 ml IB, supplemented with maltose (15 mM final concentration), and induced as described above. Four successive inductions could be made on the same pellet. The combined haemolytic material (about 240 ml) contained 3–5 × 10⁵ haemolytic units (HU) of SLS obtained from 2.5 l of culture [450–750 HU (mg cell dry wt)⁻¹].

Streptolysin S assay. The lytic effect of SLS on erythrocytes was used for toxin assay. Defibrinated sheep blood (Institut Pasteur Production) was centrifuged (5000 g, 5 min) and the erythrocyte pellet was washed three times in 150 mM-sodium PBS, pH 6.8 (Alouf & Raynaud, 1968). Sheep red blood cells (SRBC) were suspended (about 2.5%, v/v) in PBS such that a 30-fold dilution of this suspension in distilled water gave an A_{541} of 0.200. This standard SRBC suspension (about 6 × 10⁸ cells ml⁻¹) was kept at 4 °C and used within 5 d.

Volumes of appropriately diluted toxin solution decreasing in 0.1 ml amounts from 1 to 0.1 ml were placed in tubes and the volume in all tubes was brought to 1 ml by adding PBS; then 0.5 ml SRBC was added. The tubes were incubated at 37 °C for 45 min and then briefly centrifuged. The percentage of haemolysis was estimated by the A_{541} of the haemoglobin in the supernatant fluid. One HU was defined as the amount of test material which caused 50% lysis of the cells. Values were estimated graphically by plotting on a log probit graph (Alouf & Raynaud, 1968) the percentage of lysis against the volume of toxin sample. Appropriate controls were set by mixing the test material with cholesterol (10 µg ml⁻¹), which inhibits the haemolytic activity of SLO but not that of SLS, or with trypan blue (13 µg ml⁻¹), which inhibits SLS. The RNA-core alone used for induction did not exhibit any haemolytic activity up to 10 mg ml⁻¹.

Effect of various compounds on haemolytic activity. Preparations of SLS and BHF (see below) with an initial haemolytic activity of 10 HU were used. The potentially inhibitory compounds were used at the following concentrations (the time of preincubation, if any, with the toxin before adding SRBC is shown in parentheses): bovine serum albumin, 0.5% (w/v); sucrose, 0.3 M; Ca²⁺, 5 mM; phosphatidylcholine, 4 mg ml⁻¹ (10 min); trypan blue, 13 µg ml⁻¹ (10 min); cholesterol, 100 µg ml⁻¹ (10 min); enzymes (see Results; 1 enzyme unit per HU, 2 min). Compounds were considered inhibitory if they inhibited lysis by at least 80%.

Protein determination. Protein content was determined by the method of Lowry. Bovine serum albumin was used as a standard.

Slab PAGE. SLS preparations (20–100 µg protein ml⁻¹) were analysed by SDS-PAGE according to Laemmli (1970). Toxin samples (20–50 µl) containing 1.5% (w/v) SDS, 10% (v/v) glycerol, and 5% (v/v) 2-mercaptoethanol in Tris/glycine buffer were boiled for 90 s in a water bath before loading onto the gel. The tracking dye (0.001% bromothymol blue) was added only to the M_r markers. Electrophoresis was run on a 15% (w/v) polyacrylamide

slab gel (pH 8.9) (10 × 14 cm; 0.75 mm thick) at 2.5 mA for 15 h until the dye had migrated to 1 cm from the lower edge of the plate. Sample wells were made from 3% (w/v) acrylamide containing 0.1% SDS. After electrophoresis the gels were silver stained (Morrissey, 1981).

The same preparations were also analysed by electrophoresis at 4 °C under non-denaturing conditions, by omitting SDS and 2-mercaptoethanol.

Isoelectric focusing (IEF). This was done with a 110 ml column (LKB) as directed in the LKB manual. A pH 3.5–11 gradient was generated with two sucrose solutions containing respectively 5 and 15% (v/v) carrier ampholytes (LKB Ampholines) and 1.5 and 45% (w/v) sucrose. The less dense solution contained the toxin sample (300 µl, 40 000 HU). The central electrode solution (anode) consisted of 1% (v/v) sulphuric acid and 60% (w/v) sucrose. The cathode solution (0.2 ml ethylenediamine in 10 ml distilled water) was layered on the top of the gradient. A final potential of 1600 V was applied for 16 h at 2 °C, then the column contents were collected (1 ml fractions) and monitored for pH (4 °C), A_{280} and haemolytic titre. When specified, IEF under dissociating conditions was run in the presence of 6 M-urea dissolved in the sucrose solutions.

Purification of streptolysin S. All steps were done at 4 °C. Crude non-dialysed SLS preparation (240 ml) was applied to a column (2.5 × 14 cm) of hydroxylapatite that had been equilibrated with 100 mM-potassium phosphate/100 mM-ammonium acetate buffer (pH 7.0). The column was washed with this buffer and then eluted with 400 mM-potassium phosphate/100 mM-ammonium acetate buffer (pH 7.0). The flow rate was 30 ml h⁻¹ and 3 ml fractions were collected and analysed for A_{280} and haemolytic titre. The haemolytically active fractions were pooled (about 30 ml) and concentrated by ultrafiltration in an Amicon cell fitted with a YM2 membrane (M_r cut-off 1000). After repeated washing in this cell with 20 mM-potassium phosphate/100 mM-ammonium acetate buffer (pH 7.0), the retentate (fraction F1, 4.5 ml) was supplemented with 5 ml 6 M-guanidine. HCl as described by Lai *et al.* (1978), allowed to stand at room temperature for 15 min, ice-chilled, loaded onto a Sephadex G-100 column (2.6 × 100 cm) equilibrated with 20 mM-potassium phosphate/100 mM-ammonium acetate buffer (pH 7.0) and eluted (flow rate 15 ml h⁻¹) with this buffer. Eluted fractions (3.5 ml) were monitored as described above. The selected haemolytic pool was concentrated by ultrafiltration (fraction F2, 2.2 ml) and stored at -20 °C.

Amino acid analyses. These were done by the method of Spackman *et al.* (1958) with a Beckman Multichrom B analyser, using the monocolumn procedure of Devenyi (1969). Samples of purified SLS (10 µg) and RNA-core (10 µg) were dialysed against water and then hydrolysed for 24, 48 or 72 h at 110 °C with 6 M-HCl in sealed Pyrex tubes after evacuation and degassing. The hydrolysates were dried under air before analysis.

Determination of the M_r of denatured basic haemolytic fraction (BHF). This fraction (1 ml, 1000 HU) separated from purified SLS by IEF was boiled for 2 min in 1.5% (w/v) SDS and loaded onto a Biogel P6 (Bio-Rad) column (2.5 × 40 cm) equilibrated with 20 mM-potassium phosphate/100 mM-ammonium acetate buffer (pH 7.0) and previously calibrated with the following peptides (boiled in 1.5% SDS): aprotinin (6 kDa), staphylococcal delta-toxin (2.9 kDa), bovine chain B insulin (3.3 kDa) and gramicidin D (1.7 kDa). The BHF sample was eluted with the same buffer (flow rate 50 ml h⁻¹) and 1 ml fractions were monitored for A_{280} .

RESULTS

Purification of SLS

A summary of purification steps is given in Table 1. The haemolytic activity of the crude material was recovered as a single sharp peak in fractions 160–190 of the hydroxylapatite column (Fig. 1); these fractions were pooled and concentrated. The resulting preparation (fraction F1), supplemented with guanidine. HCl, was gel-filtered on a Sephadex G-100 column. Two protein peaks were eluted, the first of which coincided with haemolytic activity (Fig. 2). Fractions 80–110 had the highest specific activity. They were pooled and concentrated (fraction F2). The apparent M_r of this pool determined by gel filtration on the calibrated column was about 20 000 in the presence of guanidine. HCl and 40 000 in its absence. When submitted to SDS-PAGE and silver staining, fraction F2 ran as a single band (Fig. 3) migrating at the same R_F as bromothymol blue, suggesting an apparent M_r below 4000. Fraction F2, which contained 41% of the haemolytic activity of the initial crude material, was considered to be highly purified SLS. Its specific activity ranged from 1.2 to 3.6×10^6 HU mg⁻¹ for the various preparations purified.

Characteristics of purified SLS

When submitted to PAGE in the absence of detergent, fraction F2 revealed a single haemolytic band on the migration front, as shown either by cutting the gel with subsequent elution and titration or by layering it with SRBC (data not shown). Consequently fraction F2 appeared to be a single haemolytic entity negatively charged at pH 8.9.

Fig. 1. Chromatographic separation of crude SLS on a hydroxylapatite column (see Methods). —, A_{280} ; ----, haemolytic activity.

Fig. 2. Gel filtration of fraction F1 (Table 1) on a Sephadex G-100 column in 6 M-guanidine.HCl (see Methods). —, A_{280} ; ----, haemolytic activity.

Table 1. *Purification of SLS*

The procedure is fully described in Methods.

Fractionation step	Volume (ml)	Total activity (HU)	Total protein (mg)	Specific activity (HU mg ⁻¹)	Purification factor	Yield (%)
Starting material	240	533000	12.6	42300	1	100
Hydroxylapatite chromatography (fraction F1)	4.5	536000	0.72	745000	17.6	100
Gel filtration (fraction F2)	2.2	218250	0.185	1178000	27.9	41

Table 2. *Amino acid composition (nmol) of purified SLS (10 µg) and RNA-core (10 µg)*

Amino acid	Purified SLS	RNA-core	Amino acid	Purified SLS	RNA-core
Glu or Gln	5.9	4.6	Tyr	0.7	0.9
Asp or Asn	5.2	4.5	Phe	0.85	0.8
Gly	24.6	18.1	Lys	2.4	6.0
Ala	4.2	4.6	Arg	1.2	0.9
Val	1.7	2.2	Thr	1.7	2.8
Ile	0.85	1.0	Ser	3.1	3.5
Leu	1.5	1.5	Met	0.7	0.3

The amino acid composition of SLS and RNA-core is reported in Table 2. The SLS preparation lacked cysteine, histidine, proline and tryptophan. The data obtained indicated that a protein contaminant was present in the inducer. Whether this contaminant remained associated with RNA-core in the purified toxin awaits further investigation.

IEF of purified SLS

Purified SLS (400 µl of fraction F2 containing 40000 HU) was submitted to IEF in a 3.5–11 pH gradient. The toxin preparation was resolved into two sharp haemolytic peaks around pH 3.6 and 9.2 and four non-haemolytic peaks (pI 3.9, 4.2, 4.5, 6.0). Only 25% of the initial activity applied was recovered. However, toxin titres were very probably underestimated due to inhibitory effects of ampholines and sucrose on erythrocyte lysis.

The acidic haemolytic fraction (about 8000 HU) was washed and concentrated by ultrafiltration as described for fraction F1. The retentate (about 2 ml), when electrofocused in a 3.5–11 pH gradient in the presence of urea, yielded two peaks, of pI 4.95 and 9.7 (Fig. 4c). Under

Fig. 3. Slab SDS-PAGE of purification fractions (Table 1). From left to right: (1) starting material, (2) fraction 1, (3) fraction 2 (purified SLS), (4) standard M_r markers.

Fig. 4. (a, b) Isoelectric focusing of purified SLS (fraction 2, Table 1) in a sucrose/ampholine pH 3.5–11) gradient in the absence (a) or presence (b) of 6 M-urea. (c) IEF in 6 M-urea of the material of the pI 3.6 peak in (a). The arrows indicate pI values at maximum A_{280} of the resulting peaks.

Fig. 5

Fig. 6

Fig. 5. Residual haemolytic activity of BHF (carrier-free SLS peptide, 2 HU ml⁻¹) as a function of incubation time at 37 °C in PBS (A) and in PBS containing 6 µM-bovine serum albumin (B), 6 µM-RNA-core (C) or 20 µM-RNA-core (D).

Fig. 6. Kinetics of haemoglobin release by SLS (----) and BHF (—). The haemolysins (100 µl, 20 HU ml⁻¹) were added to a series of tubes containing 0.9 ml PBS (pH 6.8) and 0.5 ml of SRBC and incubated at 37 °C. At the times shown the tubes were centrifuged at 4 °C (10000 g, 5 min) and haemoglobin in the supernate was assayed as described in Methods.

the same dissociating conditions, fractions F2 (Fig. 4b) yielded three peaks, of pI 5.1, 6.5 and 9.5. The haemolytic fraction of pI around 9.2 (Fig. 4a) was concentrated and washed in the Amicon cell to eliminate sucrose and ampholytes. The retentate constituting this basic haemolytic fraction (BHF) contained about 5% (about 2000 HU) of the total haemolytic material submitted to IEF. SDS-PAGE of BHF did not allow accurate determination of its M_r , because it migrated close to the tracking dye. BHF was then denatured and gel-filtered on the calibrated Biogel P6 column (1000–6000 M_r range); it emerged as a single peak of M_r about 1800 (data not shown).

Comparison of the properties of SLS and BHF

The most striking feature of BHF was its high thermal lability as compared to SLS. The half-life (in terms of haemolytic activity) at 37 °C was about 7 min for the former (Fig. 5) and 25 min for the latter. The half-life of BHF increased fivefold when RNA-core was present during incubation at 37 °C (Fig. 5). BHF was much more stable at –20 °C, but even at this temperature, a progressive loss of haemolytic activity occurred within a few weeks. Haemolytic activity was inhibited identically for both SLS and BHF upon incubation with trypan blue or phosphatidylcholine but not with cholesterol or bovine serum albumin. SLS and BHF haemolytic activities were lost after incubation with α -chymotrypsin, pronase and proteinase K. In contrast, the two haemolysins were insensitive to trypsin, carboxypeptidases A and B, and leucine aminopeptidase.

The kinetics of the haemolysis of SRBC by SLS and BHF is shown in Fig. 6. For the same amounts of haemolysins (in terms of HU), the lag preceding haemoglobin release and the time required for optimal lysis was twice as high for BHF. The effect of temperature on the lytic process was identical for both SLS and BHF. No lysis took place at 4 °C even after several hours incubation (2 HU ml⁻¹) with SRBC. The behaviour of the two haemolysins was similar as regards the effect of pH on the lytic process at 37 °C: lytic activity was maximal at pH 6.8, decreasing rapidly on the acidic side to less than 10% at pH 5.5, and more slowly on the alkaline side up to pH 9.0. The haemolytic activity of SLS and BHF was optimal in 75 mM-sodium phosphate/75 mM-NaCl at pH 6.8. For both preparations, it decreased at the same pH by 20% in 150 mM-sodium phosphate, 60% in 150 mM-potassium phosphate and 40% in 19 mM-citric acid/131 mM-sodium phosphate. BHF activity (1.5 HU ml⁻¹) was totally inhibited by 5 mM-Ca²⁺ and by 70% by 5 mM-Cu²⁺; the inhibition was 80% and 60%, respectively, for SLS. Neither haemolysin was inhibited by 5 mM-Zn²⁺ or by 5 mM-Mg²⁺. Both SLS and BHF were inhibited by 0.3 M-sucrose.

DISCUSSION

SLS is unusually labile (Bernheimer, 1983) and its instability through manipulative steps, probably due to its hydrophobicity, has hampered its purification and characterization (Ginsburg, 1970; Lai *et al.*, 1978). No indication as to the molecular homogeneity of purified SLS preparations has been provided by previous investigators and most efforts have been directed toward the isolation of the carrier-haemolysin complex rather than that of the putative haemolytic peptide moiety (see Introduction). In the present work we devised a procedure for obtaining high yields of SLS [about 2×10^5 HU (l culture)⁻¹] by repetitive induction of the same suspension of streptococcal cells with RNA-core. The toxin was purified to apparent electrophoretic homogeneity by a simple two-step process with good recovery (40%). Both induction and hydroxylapatite column elution buffers contained the same phosphate concentration, thus allowing direct loading of crude SLS without prior dialysis or lyophilization, which cause significant loss of toxin.

The purified toxin was visualized for the first time by silver-stained SDS-PAGE. By this technique the M_r was estimated as below 4000, whereas we obtained a value of 40000 by gel filtration on Sephadex G-100 (as was also reported by Hryniewicz *et al.*, 1978) and of 20000 by the same technique in 6 M-guanidine. In similar determinations in guanidine or urea, values of 15200 and 22500 were reported by Lai *et al.* (1978) and Hryniewicz *et al.* (1978), whereas a value of 18500 was obtained by Calandra & Oginsky (1975) in sucrose buffer. Bernheimer (1967) reported a value of 20000, obtained by ultracentrifugation in a sucrose gradient. These data suggest a tendency of SLS to aggregate, as also observed for two membrane-damaging hydrophobic peptide toxins, staphylococcal delta-toxin (Freer & Arbuthnott, 1983) and bee venom melittin (Talbot *et al.*, 1982).

Purified SLS (fraction F2) had a specific activity of 3×10^6 HU (mg protein)⁻¹ determined according to the assay system of Lai *et al.* (1978), a value similar to that reported by these investigators. On the basis of these data SLS is one of the most powerful haemolytic agents known (see Alouf, 1977). Its activity is about 100000 times that of surfactin, saponin, melittin and staphylococcal delta-toxin (Bernheimer, 1972, 1983).

The amino acid composition of purified SLS showed some similarities with that reported by Koyama (1963) and Lai *et al.* (1978) as regards the proportions or the absence of certain residues. However, interpretation of these data is difficult because of the presence of contaminating protein(s) in RNA-core, also reported in the inducing ribonucleotides used by Koyama (1963) and Lai *et al.* (1978). A preliminary attempt to sequence purified SLS obtained in this work failed, as did amino terminal determination by Edman degradation, suggesting that this residue might be blocked, as found for staphylococcal delta-toxin (Fitton *et al.* 1984), or that the peptide moiety of SLS could be cyclic, like various ionophores and membrane-damaging peptides such as gramicidin (Fornili *et al.*, 1984) and *Microcystis aeruginosa* toxin (Grabow *et al.*, 1982).

Purified SLS was submitted to IEF in an attempt to isolate the peptide moiety and to investigate whether the RNA contaminant protein was associated with the toxin. SLS resolved into six peaks of different pI, two of which, of pI 3.6 and 9.2, were haemolytic. The major haemolytic activity was located in the acidic fraction. This probably corresponds to undissociated RNA-SLS complex, which would therefore appear as a highly negatively charged molecule (as also inferred from the migration of the toxin when submitted to PAGE in non-dissociating conditions at pH 8.9). The negatively charged repeating polymer backbone of the ribonucleotide core might contribute significantly to the net charge of the toxin. The same acidic haemolytic fraction when refocused in urea split into two components, of pI 4.95 and 9.7, whereas under the same conditions fraction F2 yielded three peaks, of pI 5.1, 6.5 and 9.5, suggesting that the material focusing around pH 6.0 was RNA-core contaminating protein(s). The pI 3.6 fraction was free from this material and thus more pure than F2. The haemolytic fraction of pI 9.2 (BHF) is apparently the peptide moiety of SLS isolated for the first time in a haemolytically active state, as discussed below. The apparent M_r of this fraction denatured by heating with SDS was 1800.

BHF and purified SLS shared common properties as regards haemolytic activity. However, BHF was much more labile than SLS and was substantially stabilized by RNA-core as a

function of the concentration of this component up to a critical value. Above this value a decrease of BHF stability was observed, suggesting that the effect of RNA-core could not be simply attributable to non-specific protection of BHF against adsorption to surfaces. The kinetics of the lysis elicited by BHF was similar to that found for SLS except for the longer lag period. This may either be due to the higher lability of the peptide or to carrier involvement in cell-toxin interactions.

SLS-like toxins have been shown to be produced by *Treponema hyodysenteriae* in the presence of RNA-core, Tween 80 and serum albumin (Knoop, 1981; Lemcke & Burrows, 1982), by *Streptococcus mutans* in the presence of Tween 80 (Woltjes *et al.*, 1981) and by *Streptococcus agalactiae* in the presence of Tween, starch or lipoteichoic acid (Marchlewicz & Duncan, 1980; Dal & Monteil, 1983; Tsaihong & Wennerström, 1983). For the last two organisms RNA-core was inactive as an inducer. A comparative study of these various haemolysins would be of great interest. The demonstration of SLS production *in vivo* (Duncan, 1983) and the known leucotoxicity of the toxin (Sullivan & Mandell, 1981) warrants further study of SLS and related toxins and their possible contribution to pathogenicity. The work described here, particularly the isolation of the haemolytic peptide, will enable us to gain a better insight into SLS structure, its cytolytic effects and the biological relation between the peptide and the inducer/stabilizer moiety.

We thank Drs A. M. Gilles, P. Falmagne and A. de Wolff for assistance with protein analysis, Drs C. Geoffrey and C. Jolivet-Reynaud for helpful discussions and Miss L. Cayrol for secretarial assistance.

This work was supported by grants from AGIR and the Caisse Nationale de l'Assurance Maladie des Travailleurs Salariés, which we are pleased to acknowledge.

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