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NOTE

Separation of Gamma Hemolysin from *Staphylococcus aureus* Smith 5R

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Staphylococcus aureus strain Smith 5R was found to produce a hemolysin with an isoelectric point of 9.5 and with a hemolytic spectrum different from alpha, beta, and delta hemolysins.

Alpha, beta, and delta hemolysins of *Staphylococcus aureus* have been purified and studied extensively (2–4, 6, 8) during the last decade. The existence of a gamma (12) and an epsilon hemolysin (4, 5) from staphylococci was reported many years ago, but their existence was not previously convincingly established (4). Gamma lysin was differentiated from alpha lysin antigenically (9), and Smith isolated a strain producing gamma lysin (11). Guyonnet and Plommet recently showed that strain Smith 5R produces two hemolytic factors which act synergistically and have different properties from alpha, beta, and delta lysin (7).

Strain Smith 5R was cultivated for 24 hr in 100 ml of Casein Hydrolysate-Yeast Extract medium in ordinary 1-liter Erlenmeyer flasks. No special arrangements were made for active aeration. The flasks were inoculated and placed on a shaking table at 37 C. The cultures were centrifuged (8,000 × *g*, 10 min; cell dry weight, 4 to 6 g/liter) and assayed for hemolytic activity as previously described (14). The supernatants contained about 640 hemolytic units (HU)/ml against rabbit red blood cells and 40 HU/ml against human cells. The hemolytic activity in cultures contained in flasks with indentations or active aeration, or both, was very low (<10 HU/ml, human red blood cells).

Fifty milliliters of crude supernatant was dialyzed against glycine (1%, w/v) and subjected to isoelectric focusing in a 110-ml column (LKB-Produkter, Stockholm-Bromma, Sweden; reference 13). Each fraction was assayed against rabbit and human red blood cells. Three adjacent fractions, *p*H 8.5 ± 0.1 ("peak 8.5"), contained a hemolysin preferably lysing the rabbit cells; less than one-tenth of the activity was found when assayed against human or horse cells under identical conditions.

Fractions of a second peak (*p*H 9.5 ± 0.1, "peak 9.5") were more active than peak 8.5 fractions against human cells. It could thus be suspected that peak 9.5 was contaminated by delta lysin (isoelectric point, *p*I 9.6; reference 10). However, it had a different hemolytic spectrum compared with purified alpha, beta, and delta lysins (Table 1). No synergism or antagonism between the 8.5 and the 9.5 hemolysin was observed.

The hemolysin peak 9.5, which was suspected to be identical with the gamma lysin of strain Smith 5R, was inactivated by heating at 60 C for 10 min, whereas delta lysin was not affected by this treatment. Addition of swollen agar (0.1%, final concentration; Bactoagar, Difco) to each test tube in the hemolytic assay containing rabbit or human cells lowered the titer of a sample of 640 HU/ml more than 80%. Alpha and delta lysins of similar concentrations were unaffected; beta lysin (10^9 HU/ml, sheep red blood cells) was also unaffected. Thus, the inhibition seems unique for this new staphylococcal hemolysin. The activity was inhibited to the same extent by different batches of Difco agar, but it was inhibited less by Noble agar (Difco) and not at all by 0.1% purified agarose (l'Industrie Biologique Francaise, S. A. Gennevilliers, Seine, France). This probably means that the basic gamma lysin is inhibited by acidic groups of the agar polymer or by impurities in the preparations.

Attempts to prepurify gamma hemolysin (*p*I 9.5) by ion-exchange chromatography were made. The toxin was not adsorbed on CM-Sephadex C-25 under conditions when delta lysin is adsorbed (10). However, DEAE-Sephadex A-25

TABLE 1. *Sensitivity to red blood cells from different species*[a]

Species	"Peak 9.5"	"Peak 8.5"	Alpha[b]	Delta[c]	Beta[d]
Rabbit	10,240	10,240	10,000	40	10^2
Human	640	<10	<10	80	10
Sheep	2,560	160	100	10	10^3
Goat	2,560	160	100	20	10^5
Dog	640	640	100	160	10
Chicken	<10	10	10	<10	10
Horse	20	<10	<10	160	<10

[a] Results expressed as hemolytic units per milliliter.
[b] Alpha hemolysin, α_{Ia}; pI 8.5 (14).
[c] Delta hemolysin highly purified, kindly supplied by A. Kreger.
[d] Beta hemolysin highly purified (Wadström and Möllby, *in preparation*).

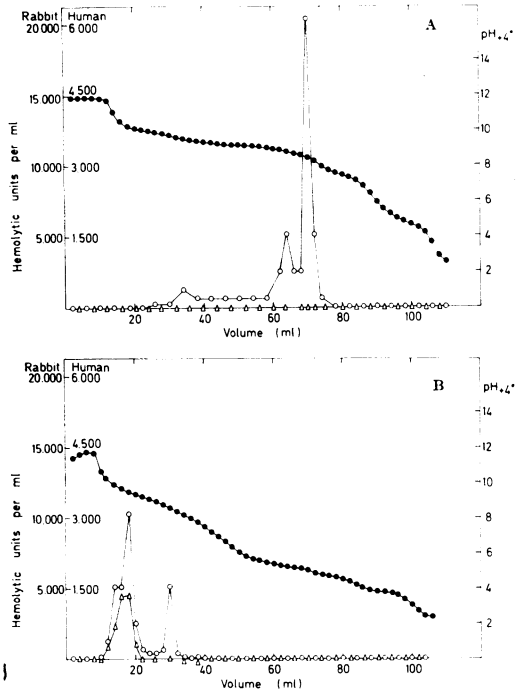

FIG. 1. *Isoelectric focusing of the effluent after diethylaminoethyl (DEAE) Sephadex batch adsorption of staphylococcal culture supernatant fluid (Fig. 1A). DEAE Sephadex (10 g, dry weight) was equilibrated against 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, (pH 8.5) and mixed with 700 ml of dialyzed (15) crude culture supernatant fluid for 2 hr at 4 C. The eluent obtained by treating the DEAE Sephadex for 20 min with 0.2 M Tris-hydrochloride (pH 8.5) supplemented with 0.2 M NaCl was dialyzed against 1% (w/v) glycine and also separated (Fig. 1B). Isoelectric focusing was performed in a glycerol density gradient (20 to 70%, v/v) containing 1.0% (w/v) Ampholine at 500 to 600 v and 1 to 2 ma for 48 hr. Fractions of 2 ml were collected, the pH was recorded at 4 C (●), and each fraction was assayed for hemolytic activity against rabbit (○) and human (△) red blood cells.*

adsorbed the gamma lysin in crude culture supernatant after dialysis (15), as shown by isoelectric focusing of the effluent and eluent (Fig. 1A and 1B). It is remarkable that an alkaline protein is adsorbed and eluted from an anion exchanger. This is another unique property of this hemolysin; the basic proteins alpha, beta, and delta lysins are not adsorbed (10). The adsorption might be due to a complex formation between the basic protein and acidic material. This aggregate was then split upon elution, since separation by isoelectric focusing of the DEAE eluent gave a peak of *p*I 9.5, whereas peak 8.5 had nearly disappeared (Fig. 1B). The latter hemolysin, which is most active against rabbit red blood cells, was found in the DEAE effluent (Fig. 1A) and probably corresponds to the main component of alpha hemolysin (α_{Ia}) previously separated by isoelectric focusing (13, 14).

For several reasons, it is obvious that the hemolysin with a *p*I of 9.5 from *S. aureus* strain Smith 5R is probably not related to alpha, beta, or delta hemolysins. Work is now in progress to purify "gamma, *p*I 9.5" by ion-exchange chromatography, isoelectric focusing, and molecular sieve chromatography.

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