AN INHIBITOR PRODUCED BY A STRAIN OF STAPHYLOCOCCUS HOMINIS FROM NORMAL HUMAN SKIN

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It is not certain whether any benefit may be derived from carriage of "antibiotic" producing skin bacteria (Noble and Willie 1980) but the purified substances may be of therapeutic value (Selwyn et al 1975).

We isolated a strain of Staphylococcus hominis which inhibited the in vitro growth of many Gram positive organisms including some strains of Staphylococcus aureus. Inhibitor production commenced in the exponential growth phase in liquid and solid media. The inhibitor was destroyed by heating at 75°C but was insensitive to protease, trypsin and chymotrypsin.

Titre was calculated as the reciprocal of the highest doubling dilution causing inhibition on an indicator lawn and was expressed in arbitrary units (AU) ml^{-1}. Optimum titre was achieved by growing the producer strain in semi-solid (0.2% agar) Tryptone Soya Broth, pH 6.5, in a shaking flask incubator at 34°C. Freezing and thawing the medium after 24h growth followed by centrifugation gave a crude extract (CE) of inhibitor in the supernatant. CE was treated with 45% ammonium sulphate and the redissolved precipitate was dialysed against 0.05M Tris HCl buffer pH 6.5 using an inner dialysis bag containing polyethylene glycol 6000 by a modification of the method of Kohn (1959). Using the same buffer, dialysed CE was eluted through a Sephadex G75 Superfine column; all inhibitory activity was in the void volume indicating a molecular weight $\geqslant$ 70,000. This purified inhibitor, (PI), adsorbed onto sensitive cells and was bactericidal.

At all stages of purification, titre increased markedly on storage; this could indicate breakdown of the inhibitor to active subunits. To investigate, PI was eluted through a Sephacryl S200 SF column calibrated with known molecular weight proteins. The elution profile showed inhibition in all fractions from molecular weight c. 200,000 - 70,000. Polyacrylamide gel electrophoresis (PAGE) of PI produced a single strong protein band which caused inhibition on bioassay. Sodium dodecyl sulphate/PAGE of PI revealed a similar protein band with an Rf value corresponding to a molecular weight of c. 35,000. The apparently high molecular weight of the inhibitor on gel filtration was probably due to aggregation of active subunits or binding of the inhibitor to inert molecules.

Table 1: Analysis of initial (and 72h) titres of inhibitor to show effect of storage.

Sample	Total protein (mg)	Total AU inhibitory activity	Specific Activity (AU mg^{-1} protein)
CE	2040	4800 (14400)	2.4 (7.1)
Ammonium sulphate precipitate	1025	3200 (6400)	3.1 (6.2)
Dialysed sample	659	4096 (6144)	6.2 (9.3)
PI	171	2880 (5760)	16.8 (33.7)

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