Induction of Tetracycline Resistance in *Staphylococcus aureus* in the Absence of Lipid Synthesis

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INTRODUCTION

Resistance to tetracycline in *Staphylococcus aureus* and enteric bacteria is often plasmid determined. The characteristics of plasmid-mediated tetracycline resistance in these bacteria are similar, in that resistance is usually inducible (Izaki, Kiuchi & Arima, 1966; Franklin, 1967; Sompolinsky *et al.* 1970*a*; Franklin & Cook, 1971; Chopra, Lacey & Connolly, 1974; Foster & Walsh, 1974; Shipley & Olsen, 1974; Asheshov, 1975), and appears to result from decreased uptake of the antibiotic (Izaki *et al.* 1966; Franklin, 1967; De Zeeuw, 1968; Sompolinsky *et al.* 1970*a*; Avtalion *et al.* 1971; Kuck & Forbes, 1973; Shipley & Olsen, 1974).

Although the precise mechanism of tetracycline resistance is unknown, these features suggest that the gene products involved in resistance may be similar in all cases. This view is supported by the data of Levy & McMurry (1974) and Boldur & Sompolinsky (1974) which indicate that in enteric organisms at least, expression of tetracycline resistance following induction probably results from the insertion of a protein (or proteins) into the envelope. In *S. aureus*, a protein (or proteins) associated with the expression of tetracycline resistance has also been found (Avtalion *et al.* 1971), but this is undetectable in membrane preparations (Chopra *et al.* 1974). However, it seems probable that this protein is indeed associated with the staphylococcal membrane *in vivo* and may be released during membrane isolation (Chopra *et al.* 1974). This view is supported by the findings of Boldur & Sompolinsky (1974) which suggest that the proteins associated with tetracycline resistance may only be loosely associated with the membrane in certain organisms.

Studies on the assembly of bacterial membranes have shown that some proteins can be incorporated into the membrane in a functional form in the absence of lipid synthesis (Machtiger & Fox, 1973; Randall, 1975). Since expression of plasmid-mediated tetracycline resistance probably results from the insertion of proteins into the membrane, it is of interest to know whether or not resistance can be expressed in the absence of lipid synthesis.

[METHODS]

Bacteria. *Staphylococcus aureus* strain UB4012, which contains plasmid pUB111 (specifying resistance to tetracycline), has been described previously under the former description 649 *tet-r*(p) (Chopra *et al.* 1974).

Culture media. These have been described previously (Chopra *et al.* 1974).

Other materials. Cerulenin was the gift of S. Ōmura. L-3-[G-³H]phenylalanine (1 mCi/μmol) and uniformly-labelled sodium [¹⁴C]acetate (59 μCi/μmol) were obtained from the Radio-

chemical Centre, Amersham, Buckinghamshire. Tetracycline hydrochloride (achromycin) was purchased from Lederle Laboratories, London.

Estimation of fatty acid synthesis in whole bacteria. The incorporation of radioactivity from sodium [^{14}C]acetate into chloroform-extractable material was used as a measure of fatty acid synthesis. Bacteria were grown in the presence of uniformly-labelled sodium [^{14}C]acetate (0.2 $\mu\text{Ci/ml}$) and 0.8 ml samples of culture extracted at 4 °C by the method of Bligh & Dyer (1959). Samples (0.5 ml) of the chloroform phase (containing extracted lipids) were evaporated to dryness in scintillation vials and 5 ml of a scintillation fluid (see Lacey & Chopra, 1972) added. Radioactivity was estimated in a Packard Tri Carb liquid scintillation spectrometer.

Estimation of protein synthesis in whole bacteria. Bacteria suspended in a nutrient broth containing L-3-[G- ^3H]phenylalanine (2 $\mu\text{Ci/ml}$) were incubated with shaking at 37 °C for various periods. Growth was terminated by the addition of trichloroacetic acid to 5% (w/v) and incorporation of radioactivity into protein determined as previously described (Lacey & Chopra, 1972).

RESULTS

Cerulenin [(2S) (3R) 2,3,-epoxy-4-oxo-7,10-dodecadienoylamide] is a specific inhibitor of β -keto acyl thioester synthetase and therefore interferes both with *de novo* fatty acid synthesis and with chain elongation from palmityl CoA (D'Agnolo *et al.* 1973). Addition of cerulenin (125 $\mu\text{g/ml}$) to growing cultures of UB4012 resulted in virtually complete inhibition of fatty acid synthesis (Fig. 1*a*), but had little effect on protein synthesis during a period of 40 min following addition of the antibiotic (Fig. 1*b*). These results are essentially similar to those obtained by Goldberg, Walker & Bloch (1973) and Randall (1975), who demonstrated that concentrations of cerulenin sufficient to inhibit lipid synthesis had little immediate effect on protein synthesis in *Escherichia coli*.

Pre-incubation of UB4012 for 40 min with 5 μg tetracycline/ml produced a marked fall in the subsequent inhibition of protein synthesis by the antibiotic, compared with organisms grown initially in the absence of drug (Fig. 2). These results are essentially the same as those described previously (Chopra *et al.* 1974) and represent induction of tetracycline resistance.

Addition of cerulenin (125 $\mu\text{g/ml}$) during pre-exposure of UB4012 to tetracycline (5 $\mu\text{g/ml}$) had no effect on the subsequent degree of inhibition of protein synthesis by tetracycline compared with organisms pre-incubated in the presence of tetracycline, but without cerulenin (Fig. 2). Thus, induction of tetracycline resistance appears to occur to the same extent in the absence of lipid synthesis as under normal growth conditions.

DISCUSSION

Synthesis of membrane proteins in the absence of lipid synthesis could lead to at least three possibilities with respect to their association with the membrane: (i) lack of incorporation into the membrane, (ii) incorporation into the membrane, but in a manner resulting in impairment of functional capacity of the protein, and (iii) integration and correct insertion into the membrane, resulting in full functional capacity. Although there appear to be no examples of membrane proteins that cannot be incorporated into membranes in the absence of lipid synthesis, examples of the situations arising in (ii) and (iii) are known (Machtiger & Fox, 1973; Randall, 1975).

The data presented here suggest that tetracycline resistance in *S. aureus* can be induced in the absence of lipid synthesis. If, as seems probable, resistance results from insertion of

Fig. 1. Effect of cerulenin on (a) lipid synthesis and (b) protein synthesis in UB4012. Either (a) uniformly-labelled sodium [^{14}C]acetate ($0.2 \mu\text{Ci/ml}$) or (b) L-3-[G- ^3H]phenylalanine ($2 \mu\text{Ci/ml}$) were added at time zero to exponentially-growing cultures of UB4012 (10^8 bacteria/ml). After 10 min, cerulenin ($125 \mu\text{g/ml}$) was added to portions of the labelled bacteria (arrowed) and portions removed at the times indicated for estimation of radioactivity incorporated into lipid and protein in the control (●) and cerulenin-treated (○) cultures.

Fig. 2. Effect of cerulenin on induction of tetracycline resistance in UB4012. Exponential-phase cultures of UB4012 (10^8 bacteria/ml) were pre-incubated for 40 min at 37°C with (□) tetracycline (5 $\mu\text{g/ml}$), with (■) tetracycline (5 $\mu\text{g/ml}$) + cerulenin (125 $\mu\text{g/ml}$), with (●) cerulenin (125 $\mu\text{g/ml}$), or (○) with no additions. Bacteria were harvested by centrifugation (15000 g, 10 min, 4°C), washed twice in cold nutrient broth, and resuspended in the same medium to give an E_{675} of 0.2 (equivalent to 10^8 bacteria/ml in growing cultures). Incorporation of L-3-[G- ^3H]phenylalanine (2 $\mu\text{Ci/ml}$) into protein (during 40 min) in the presence of the indicated concentrations of tetracycline was then determined for each culture.

a plasmid-specific protein into the membrane, these results imply that this process can occur normally in the absence of lipid synthesis.

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