FRACTIONATION OF ISOLATED BACTERIAL MEMBRANES

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Dissociation of isolated bacterial membranes by treatment with surface-active agents or by exposure to ultrasound has given "subunit" products which are lipid-protein or lipid-protein-surface-active agent complexes and which behave in a rather uniform manner when examined in the analytical ultracentrifuge (Brown, 1965; Razin et al., 1965; Salton and Netschey, 1965). This behavior in the ultracentrifuge masks the complexity of the protein constituents of the membranes but progress has been made recently in resolving the components in mitochondrial membranes (Green and Perdue, 1966; Takayama et al., 1964), erythrocyte (Azen et al., 1965; Schneiderman, 1965) and bacterial (Rottem and Razin, 1967; Salton, 1967) membranes by disc and starch-gel electrophoresis.

In order to study membrane proteins, we have developed two fractionation procedures involving dissociation with surface-active agents, yielding fractions which retain their ability to react with antibodies to the isolated membranes. The products have been compared with those obtained by removal of lipid with a modification of the n-butanol extraction method originally used by Morton (1950) and recently applied to erythrocyte membranes (Maddy,

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1966). Membranes used in our studies were isolated from Micrococcus lysodeikticus, Sarcina lutea, and Bacillus subtilis under the conditions outlined by Salton and Ehtisham-ud-din (1965) and the homogeneity of the preparations examined as described by Salton (1967).

Lyophilized or freshly prepared membranes were added to 0.05M tris buffer, pH 7.5, to give suspensions containing 10 mg/ml, w/v. Membranes were generally first dissociated by sonication (Salton and Netschey, 1965), although this step could be omitted when Nonidet P.40 was used. The dissociating agents used individually, were Nonidet P.40 (a nonionic surface-active product of Shell Company, active constituent of the polyoxyethylated alkyl phenol type) and sodium deoxycholate, both added to give final concentrations of 1%. After addition of the surface-active agents, the mixtures were held at 37°C for 30 minutes and 28 g solid ammonium sulfate was then added to 100 ml of each preparation and they were allowed to stand for a minimum of 1-2 hours at 4°C. In some experiments Triton X 100 (final concentration 1%) was added at this stage, to the deoxycholate preparations. Both the Nonidet P.40 and deoxycholate-Triton X 100 preparations were separated into two phases, a top floating layer and a lower aqueous phase by centrifugation (25,000 x g, 15 min. at 0°C). The aqueous phase (designated as Fraction 1) was separated from the non-aqueous portion by carefully withdrawing the underlying solution. The non-aqueous residues were then thoroughly dispersed in distilled water (one-half the volume of original mixture) and after centrifugation (25,000 x g, 20 min., 0°C) the preparations were further resolved into a "soluble" phase (designated as Fraction 2) and a deposit of water-insoluble material which constituted Fraction 3. The latter could be dissolved in buffer containing 0.5 - 1% sodium dodecyl sulfate (SDS) or in 6-8 M urea.

Absorption spectra of all fractions were obtained in a Cary 14 recording

spectrophotometer using 1 cm quartz cuvettes and blank of 0.05M tris buffer. Spectra of the three fractions obtained from M. lysodeikticus membranes with Nonidet P.40 showed a clear separation of the carotenoid and cytochrome membrane "markers" (Salton and Ehtisham-ud-din, 1965), the former being present almost exclusively in Fraction 2 and the latter in Fraction 3, as illustrated in Fig. 1. The spectrum of a Nonidet P.40, Fraction 3 containing 5.5 mg protein/

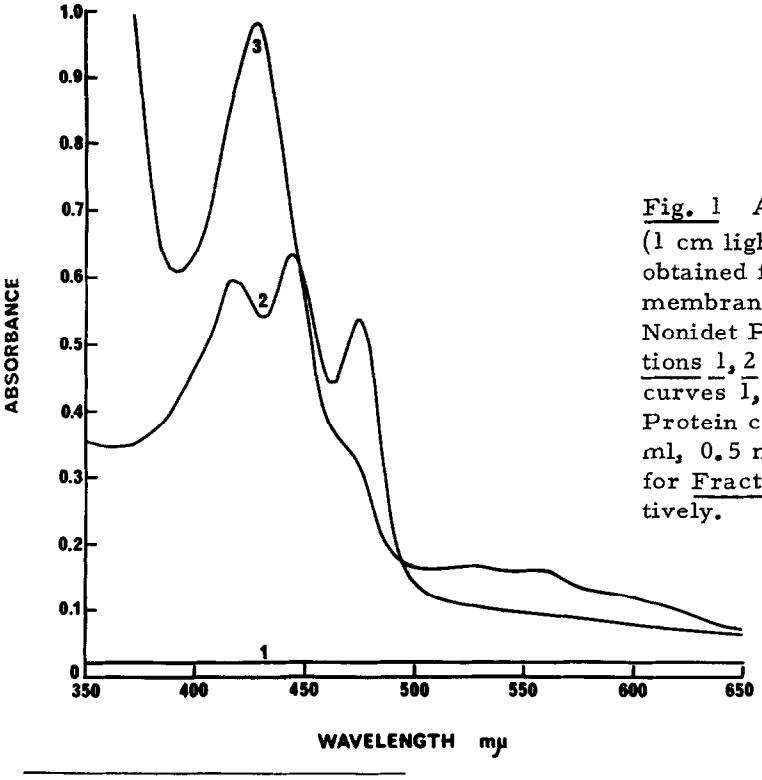

Fig. 1 Absorption spectra (1 cm light path) of fractions obtained from M. lysodeikticus membranes dissociated by the Nonidet P.40 procedure. Fractions 1, 2 and 3 are shown in curves 1, 2 and 3 respectively. Protein contents were 1.2 mg/ml, 0.5 mg/ml and 2.3 mg/ml for Fractions 1, 2, and 3, respectively.

ml, from M. lysodeikticus membranes in Fig. 2, indicates the presence of all of the original membrane cytochromes (a, b, and c) when the fraction in 1% SDS was reduced with $Na_2S_2O_4$. A similar distribution of carotenoids and cytochromes was observed when S. lutea, Nonidet P.40 fractions were examined. With the deoxycholate - Triton X 100 membrane fractions from both M. lysodeikticus and S. lutea, there was some "spreading" of carotenoids and

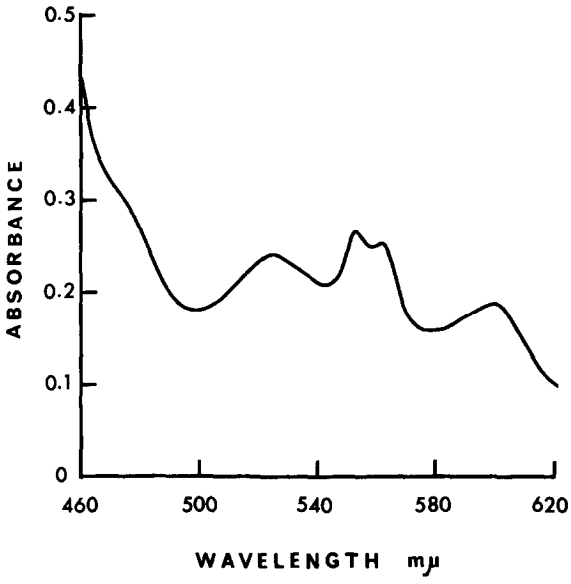

Fig. 2 Absorption spectrum of Nonidet P.40 Fraction 3 from M. lysodeikticus membranes. Fraction solubilized in 0.05 M tris buffer containing 1% SDS. The protein content was 5.5 mg/ml and the spectrum recorded in 1 cm cuvette immediately after reduction with crystals of $Na_2S_2O_4$.

cytochromes in Fractions 2 and 3. Determinations of protein after dialysis of the fractions to remove excess reagents and after precipitation of the protein in Fraction 2, gave recoveries of 80-85%. Overall recovery of phospholipid in the three fractions, using ^{32}P-labelled membranes of M. lysodeikticus, was 80-83%. The distribution of the recovered protein and ^{32}P-labelled lipid was determined for the fractionation procedures and the results are summarized in Table 1.

Disc electrophoresis of fractions dialyzed against 6M urea and run in the Model 12, Canalco equipment (Canal Industrial Corp., Rockville, Md.) was performed with either the urea-acid-gel system described by Neville (1967) or the tris-glycine buffer (prepared according to the Canalco formulation) containing 8M urea and 0.005M EDTA. The urea-acid gels were usually run at 1.5 ma per column for approximately 150 minutes and the tris-glycine urea gels at 2 ma per column for about 100 minutes. The gels were stained with aniline blue black and the behavior of Fractions 1, 2, and 3 from the Nonidet

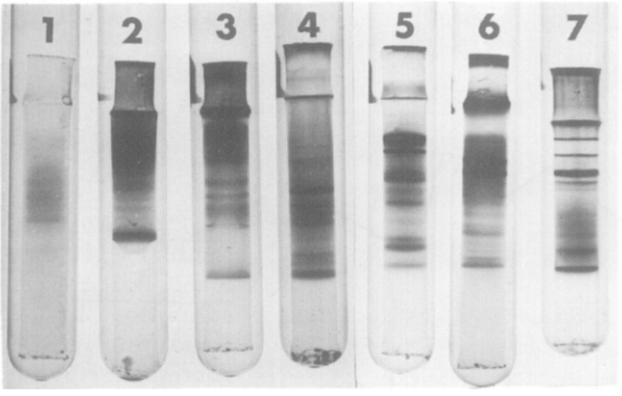

Fig. 3 Disc electrophoresis in 7% polyacrylamide gels. M. lysodeikticus membrane fractions (gels 1-6) :- Nonidet Fractions 1 (gel 1) 2 (gel 2), 2 + 1% sodium octyl sulfate (gel 3) and 3 (gel 4): acetic acid extract (gel 5); soluble fraction from butanol method (gel 6). B. subtilis soluble membrane fraction from butanol method (gel 7). Gels 1, 4, 5 and 7 run in the urea-acid system, gels 2, 3 and 6 in the tris-glycine-urea-EDTA system.

P.40 dissociated membranes of M. lysodeikticus is shown in Fig. 3 (gels 1-4). Although the patterns of bands obtained by disc electrophoresis are reproducible for a particular fractionation procedure, we have observed marked differences in components in the "soluble protein" from the n-butanol extraction method and those released by extraction with acetic acid-water (1+2, v/v/) and the resolution of some of these fractions is illustrated in Fig. 3 (gels 5-7). Thus, the fractionation procedures described in this paper have given some differential separation of membrane protein and lipid. Electrophoretic differences of components due to both differences in bacterial species and fractionation method have been observed and the complexity of the bacterial membranes confirmed.

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References

Azen, E.A., Orr, S., and Smithies, O., J. Lab. and Clin. Med., 65, 440 (1965).

Brown, J.W., Biochim. Biophys. Acta, 94, 97 (1965).

Green, D.E., and Perdue, J.F., Proc. Nat. Acad. Sci., 55, 1295 (1966).

Maddy, A.H., Biochim. Biophys. Acta, 117, 193 (1966).

Morton, R.K., Nature, 166, 1092 (1950).

Neville, D.M. Biochim. Biophys. Acta, 133, 168 (1967).

Ornstein, L., Annals of the New York Acad. of Sci., 121, 321 (1964).

Razin, S., Morowitz, H.J. and Terry, T.M., Proc. Nat. Acad. Sci., 54, 219 (1965).

Rottem, S. and Razin, S., J. Bacteriol., 94, 359 (1967).

Salton, M.R.J., and Ehtisham-ud-din, A.F.M., Aust. J. Exp. Biol. Med. Sci., 43, 255 (1965).

Salton, M.R.J., Trans, N.Y. Acad. Sci., Ser. II, 29 (1967) in press.

Schneiderman, L.J., Biochem. and Biophys. Research Communs., 20, 763 (1965).

Takayama, K., MacLennan, D.H., Tzagoloff, A., and Stoner, C.D., Arch. Biochem. and Biophys., 114, 223 (1964).