THE ISOLATION AND CHARACTERISATION OF CYTOPLASMIC MEMBRANES AND MESOSOMES OF *BACILLUS LICHENIFORMIS* 6346

D. A. Reaveley

National Institute for Medical Research, Mill Hill, London, N.W.7

Received February 14, 1968

Examination of thin sections of bacilli under the electron microscope has shown the presence of internal membrane systems as well as the cytoplasmic membrane (van Iterson, 1961; Van der Winkel and Murray, 1962; Glauert, 1962). The internal membrane complex is usually called the mesosome (Fitz-James, 1960). When cells are transferred to hypertonic solutions of substances such as sucrose or polyethylene glycol, the mesosomal membrane system appears to be extruded into the region between the cytoplasmic membrane and the cell wall in the form of small spheres (Fitz-James, 1964; Weibull, 1965). The precise relation between the spheres and the original mesosomes has not yet been unequivocably established. When the walls are removed during protoplast formation mesosomes are liberated into the supernatant fluid either as strings of beads (Ryter, Frehel and Ferrandes, 1967) or as spheres (Rogers, Reaveley and Burdett, 1967) and then could possibly be separated from the protoplasts by differential centrifugation. The present paper describes a method for the isolation of the spheres extruded from the cells and called here mesosomes, and compares their stability and protein composition with those of the cytoplasmic membranes.

METHODS

B. licheniformis 6346 taken from stationary phase cultures was washed once with buffer (0.05 M sodium hydrogen maleate-sodium hydroxide, pH 6.5,

containing 0.02 M magnesium sulphate) and polyethylene glycol 600 (20% v/v). The washed cells were resuspended in buffer to a concentration of 10 mg dry weight/ml and after the addition of lysozyme (20 µg/ml) the suspension was incubated at 35° until the rods were completely converted to round protoplasts (ca 2-3 hr). The protoplasts were collected by centrifugation (10,000 g, 1 hr, 2°). The supernatant liquid was centrifuged (100,000 g, 2 hr, 2°) and the resultant pad taken up in M-sodium chloride containing 0.02 M magnesium sulphate. Aliquots (2 ml) of the suspension were applied to caesium chloride gradients (32-34% w/v, 10 ml) containing 0.02 M magnesium sulphate. On centrifugation (100,000 g, 16 hr, 2°) two bands and a pad at the bottom of the tube formed. The upper band, which consisted exclusively of mesosomes, was removed, dialysed at 2° and freeze-dried. Yield (% cell dry wt) ca 1.8%. The lower band and the pad were isolated in a similar manner. The lower band accounted for approximately 0.6-0.9% of the cell dry weight and consisted principally of mesosomes. These mesosomes which tended to be larger than those in the upper band were often associated with amorphous material. Flagella were also present in this fraction. The pad consisted almost exclusively of ribosomes.

Protoplasts were resuspended in cold buffer to a concentration of 10 mg/ml. Two volumes of buffer without polyethylene glycol were added and a trace of DNAse. The mixture was shaken gently for 20 minutes at room temperature to lyse the protoplasts. Membranes were isolated by centrifugation (30,000 g, 20 min, 2°), washed three times with M-sodium chloride (containing 0.02 M magnesium sulphate) and three times with 0.02 M magnesium sulphate. Magnesium sulphate was removed by dialysis and the membrane preparation freeze-dried. The yield was ca 21% of the cell dry weight.

Cytoplasmic membranes and mesosomes were disaggregated by treatment with 0.1% SDS[x] in 0.1 M Tris-HCl buffer, pH 7.6, at concentrations of 5 mg/ml.

[x] SDS = sodium dodecyl sulphate

The rate of disaggregation was followed spectrophotometrically at 660 mμ. The final extent of disaggregation was determined gravimetrically after exhaustive dialysis of the supernatant and residue. Aliquots (10 μl) of disaggregated material were taken for electrophoresis.

Polyacrylamide gel electrophoresis was carried out as described by Ornstein (1964) and Davis (1964) with the exception that 0.1% SDS was incorporated into the gel solutions prior to polymerisation and into the Tris-glycine buffer (pH 8.3). The protein bands in the gels were stained with solutions of Amido Black B.

RNA was separated from DNA by the Schmidt-Thannhauser procedure as described by Hutchison and Munro (1961) and estimated by the method of Ceriotti (1955). Protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard. Lipids were removed from samples by three successive extractions (1 hr, room temperature) with chloroform/methanol (2:1 v/v).

RESULTS AND DISCUSSION

Gross chemical composition of mesosomes and cytoplasmic membranes are given in Table I.

TABLE I

The composition of cytoplasmic membranes and mesosomes of B. licheniformis 6346

	% protein	% RNA	% lipid
Cytoplasmic membranes	43-49	13-15	18-25
Mesosomes (upper band)	44[x]	2-10	17[x]

[x]Estimated in a single batch.

The upper band in the gradient separation consisted exclusively of small spheres each bounded by a unit membrane. The spheres were occasionally homogeneous in diameter (ca 600 Å) but more usually varied from 300 - 3,000 Å. In some cases mesosomes were found enclosed inside larger membranous spheres. Many mesosomes appeared to contain amorphous material inside, the nature of which is not known. The RNA content of the mesosome preparation was variable (3 - 10%) and probably reflected the extent of contamination by ribosomes (substitution of sucrose gradients for caesium chloride gradients produced only one mesosome band containing 20 - 35% RNA). Mesosomes appeared to be more stable than cytoplasmic membranes. They could for instance be mixed with PTA[x] and examined under the electron microscope without apparent disruption of the structure. Such treatment appeared to disaggregate the cytoplasmic membrane preparations.

It was found necessary when washing the cytoplasmic membrane preparations to use solutions containing magnesium ions (not less than 0.02 M) to maintain membrane integrity. Subsequent removal of magnesium ions by dialysis, however, caused no disaggregation. When examined in thin section, the membrane preparations appeared as empty sacs, many still containing a few mesosomes. Appreciable numbers of ribosomes were also present, still included within the spherical membranes; unlike mesosomes, the RNA content of membrane preparation was constant and this suggests that some ribosomes are physically attached to the cytoplasmic membranes (Abrams, Nielson and Thaemert, 1964; Nielsen and Abrams, 1964; Yudkin and Davis, 1965).

Cytoplasmic membranes (5 mg/ml) were ca 85% soluble in 0.1 M Tris-HCl, pH 7.6, containing 0.1% SDS; most mesosome preparations were completely soluble. Solubilisation was very rapid and was complete after 20 minutes at room temperature. The resulting solutions were examined by polyacrylamide gel electrophoresis.

---

[x] PTA = phosphotungstate

Four different mesosome and five different cytoplasmic membrane preparations were examined. Gels from different preparations were very similar. Some variation in pattern was occasionally encountered with different electrophoresis experiments. Photographs of gels from the same experiment are reproduced in Fig. 1 and the densitometer patterns are shown in Fig. 2.

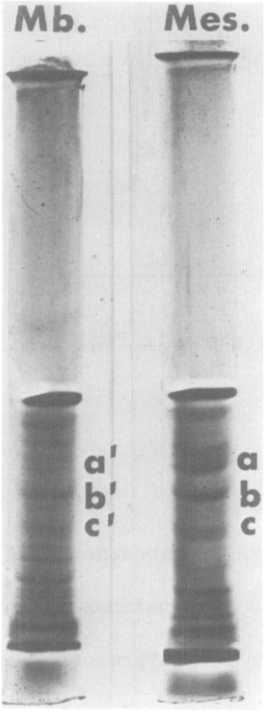

Fig. 1. Gels stained with Amido Black B. Mb = cytoplasmic membranes; Mes = mesosomes

The principal differences between membranes and mesosomes concern the regions marked A, B and C in the photographs and densitometer curves of the mesosomal proteins. Equivalent regions (A', B' and C') were present in gels from cytoplasmic membranes, but these were much less pronounced and appear in some cases to migrate more rapidly. Since the membrane preparations still contain some mesosomes, it is possible that these regions are characteristic of mesosomes. The results are not invalidated by the presence of ribosomes in the fractions for the following reasons. A mesosome preparation obtained from

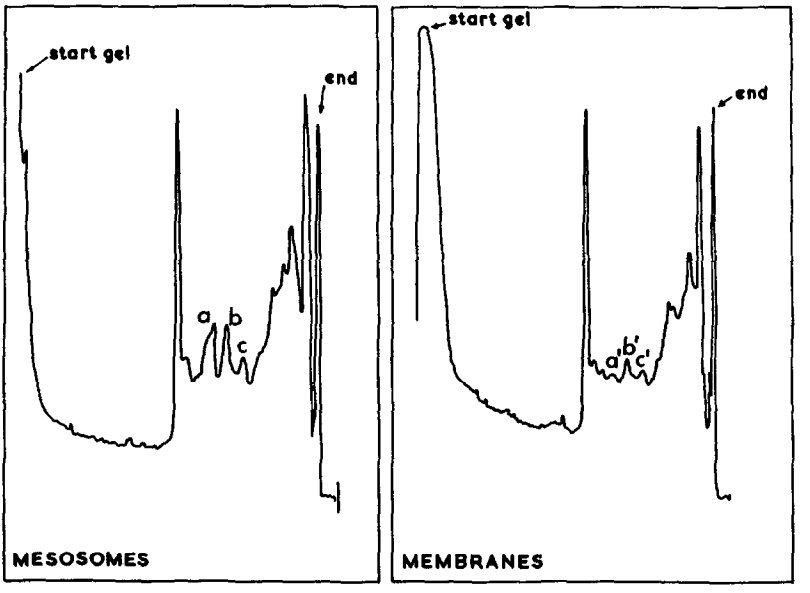

Fig. 2. Densitometer patterns for the gels shown in Fig. 1.

a sucrose gradient and thereby containing large numbers of ribosomes (RNA content 25%) yielded a protein pattern very similar to preparations isolated from caesium chloride gradients. Also the results are the reverse of what would be expected if the differences between protein patterns of mesosomes and cytoplasmic membranes were due solely to ribosomal proteins.

That mesosomes do not contain substantial amounts of soluble protein trapped inside was shown by sonication of a mesosome preparation and subsequent isolation of the insoluble residue by centrifugation. The protein pattern of the residue was very similar to that of untreated mesosomes. Patterns from lipid-extracted cytoplasmic membranes and mesosomes were identical to those not extracted. Thus two membrane systems may be isolated from B. licheniformis. The protein pattern of these membranes differs significantly.

Acknowledgements. The author wishes to thank Dr H. J. Rogers for his advice and encouragement and Mr D. B. Gunner for skilful technical assistance.

REFERENCES

Abrams, A., Nielsen, L., and Thaemert, J., Biochim. Biophys. Acta, 80, 325 (1964).
Ceriotti, G., J. Biol. Chem., 214, 59 (1955).
Davis, B. J., Ann. N. Y. Acad. Sci., 121, 404 (1964).
Fitz-James, P. C., J. Biophys. Biochem. Cytol., 8, 507 (1960).
Fitz-James, P. C., J. Bact., 88, 457 (1964).
Glauert, A. M., Brit. Med. Bull., 18, 245 (1962).
Hutchison, J. C., and Munro, H. N., Analyst, 86, 768 (1961).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem., 193, 265 (1951).
Nielsen, L., and Abrams, A., Biochem. Biophys. Res. Comm., 17, 680 (1964).
Ornstein, L., Ann. N. Y. Acad. Sci., 121, 321 (1964).
Rogers, H. J., Reaveley, D. A. and Burdett, I. D. J., XV Annual Coll. Protides of Biological Fluids, Bruges, 1967 (in press).
Ryter, A., Frehel, C., and Ferrandes, B., Compt. Rend. Acad. Sci., 265, 1259 (1967).
Van der Winkel, E., and Murray, R. G. E., J. Ultrastruct. Res., 7, 185 (1962).
Van Iterson, W., J. Biophys. Biochem. Cytol., 9, 183 (1961).
Weibull, C., J. Bact., 89, 1151 (1965).
Yudkin, M. D., and Davis, B., J. Mol. Biol., 12, 193 (1965).