The Effect of Synthetic Detergent on the Determination of the Molecular Weight of a Carotenoid-glycoprotein from *Sarcina flava*

By D. THIRKELL, M. I. S. HUNTER, J. CRAWFORD
AND A. S. FRACASSINI

Department of Biochemistry, Bute Medical Buildings, St Andrews, Fife

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SUMMARY

Membranes from *Sarcina flava* were solubilized by using 1 % (v/v) Lubrol L and a water-soluble detergent-rich carotenoid glycoprotein complex isolated and purified. Homogeneity of the complex was indicated on the ultracentrifuge but molecular weight determinations made on a membrane osmometer indicated that the molecular weight values obtained in the presence of detergent may depend on the presence or absence of salts.

INTRODUCTION

The use of synthetic detergents for the solubilization of protein complexes or of membranes is widespread (Smith, 1941; Salton & Netschey, 1965; Salton, 1967; Bailey, 1968). Since it is sometimes impossible to remove detergent from solubilized material, molecular weights have been determined in the presence of detergent (Wolken & Schwartz, 1956). The carotenoids of *Sarcina flava* are localized in the membrane (Strang, 1968) and it is likely that the dihydroxy C₅₀ carotenoid (Thirkell, Strang & Chapman, 1967) becomes an integral part of the structure of the membrane fraction. This work was to attempt to isolate and purify the complex so formed and to determine its molecular weight.

METHODS

Growth and harvesting of bacteria; preparation of carotenoid-glycoprotein. *Sarcina flava* (NCTC 7503) was grown in nutrient broth (Oxoid Ltd.) at 34° and harvested after 3 days. The membrane fraction was isolated by the method of Salton & Freer (1965) and solubilized by repeated extractions with 1 % Lubrol L (I.C.I. Ltd.) in 0.1 M-tris + maleate buffer, pH 7.2. After centrifugation at 100,000 g for 30 min. the clear yellow supernatant fluid was exhaustively dialysed against tap water followed by distilled water. The carotenoid-glycoprotein was purified by repeated precipitation with (NH₄)₂SO₄ followed by dialysis.

Ultracentrifuge for homogeneity. Solutions in distilled water or 0.1 M-KCl were exhaustively dialysed against their respective solvents for more than 48 hr. Concentrations were determined by dry weight and ash, and by refractometry.

Molecular weight determinations. Osmometry was used on both solutions in a high-speed Hewlett-Packard membrane osmometer equipped with B 19 membranes (Schliecher & Schüll). In each case, reduced osmotic pressures were plotted against concentration.

RESULTS

The complex contained carotenoid (indicated by spectrum and by reaction with SbCl_3 (Morton, 1942), sugar(s) (by the method of Whistler & Wolfrom, 1962) and a peptide (amino acid analysis). Sedimentation in distilled water or 0.1 M-KCl after 180 min. at a nominal speed of 59,780 rev./min. gave symmetrical schlieren patterns indicating a single compound characterized by a S_{20}^0 value of 1.49×10^{-13} sec.

Reduced osmotic pressure measurements recorded in the presence or absence of KCl were plotted against concentration (Fig. 1). Correction for non-ideality of solution was carried out by extrapolation to zero concentration and the results fitted into the general formula:

$$\bar{M}n = \frac{RT}{(\pi/c)_{c \rightarrow 0}},$$

where RT was corrected for the density of the solvents. From the figure, an $\bar{M}n$ value of 2.28×10^4 was obtained for the preparation in distilled water, and 6.20×10^4 for the preparation in 0.1 M-KCl. The shapes of the curves are strongly suggestive of association of macromolecules.

Fig. 1. π/C against c for (a) complex in distilled water (● -- ●), (b) complex in 0.1 M-KCl (× --- ×).

DISCUSSION

Addition of salt to a detergent-rich solution appears to bring about association of macromolecules, increasing micelle size to a point where the solubility properties of the solution tend towards those of the detergent itself. This view is supported by the discrepancy of $\bar{M}_n$ values recorded on the osmometer. Molecular weights determined in the presence of detergent may also depend on the technique used and the concentration of solution. Any molecular weight values determined in the presence of high concentrations of detergent may be open to doubt.

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