SHORT COMMUNICATION

A Water-soluble Carotenoid-glycopeptide from *Sarcina morrhuae*

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(Accepted for publication 16 March 1970)

Free pigments have been isolated from *Sarcina morrhuae* by Nandy & Sen (1967), and their results have been confirmed. When no more pigment could be extracted, the bacterial remnants were still pigmented. The present work was undertaken to see how the remaining pigment was bound.

METHODS

Sarcina morrhuae was grown on 25 % (w/v) NaCl, 1 % (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 % (w/v) $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.5 % (w/v) KCl, 1 % (w/v) yeast extract, 0.5 % (w/v) tryptone and 2.5 % agar no. 3 at 34° for 3 weeks. Pigment was removed by repeated ultrasonication in methanol and then repeated saponification with 10 % (w/v) methanolic KOH (at room temperature under an inert atmosphere and in the dark). When no further pigment was extracted, the residue was stirred at 40° in distilled water and a red-orange solution recovered.

The solution was desalted using Sephadex G75 followed by ultrafiltration in an Amicon high pressure cell. Homogeneity was tested by examining (a) sedimentation patterns in a Beckman analytical ultracentrifuge, (b) resolution on columns of (i) various grades of Sephadex, (ii) Biogel A15 (4 % agarose), and (c) resolution by disc-gel electrophoresis on 7.5 % cyanogum C40 gels at pH 4.7. An estimate of the molecular weight was obtained by (a) behaviour on dialysis (molecular weight cut-off 9000), (b) retention on columns of various Sephadexes and on 4 % agarose (standardized for both Stokes radius and molecular weight). An absorption spectrum was recorded from 210 to 600 nm. and the following were also determined: (a) dry weight and ash, (b) protein by the Folin-Lowry method using serum albumin as standard or by the quantitative ninhydrin method (Moore & Stein, 1954) with norleucine as standard, (c) carbohydrate (Whistler & Wolfrom, 1962), (d) amino acid analysis on a Technicon autoanalyser with norleucine as internal standard.

The hydrolysate prepared for carbohydrate estimation was chromatographed on Whatman no. 1 paper using either butanol + pyridine + water (6 + 4 + 3, by vol.) or ethyl acetate + pyridine + water (70 + 20 + 23, by vol.; Colombo *et al.* 1960) as solvents, with solutions of glucose, mannose, galactose and ribose as standards. Duplicate chromatograms were stained with 0.1 M-anisidine phthalate in ethanol (Pridham, 1956) or 2 % (w/v) ninhydrin in acetone.

RESULTS

The complex is a monodisperse system of molecules of low molecular weight giving a single symmetrical Schlieren pattern on the analytical ultracentrifuge. No resolution was apparent on Sephadex or on 4 % agarose. A single band was displayed on disc-gel electrophoresis with a speed of migration virtually the same as serum albumin in the system used. A molecular weight of 8000 to 9000 was suggested by (a) very slow diffusion through visking tubing, (b) no retention on Sephadex G25, but retention to an increasing degree on Sephadex G50, G75 and G100 and (c) behaviour on the 4 % agarose column, which indicated a Stokes radius of approximately 5 Å and a molecular weight of less than 10,000.

Absorption peaks were recorded at 237 and 276 nm. but their intensity was such that no fine spectrum was recorded in the visible range. The presence of carotenoid was deduced because (i) the bacterium produces red and orange free carotenoids and the complex was red-orange, and (ii) the complex gave a positive Carr-Price reaction (Morton, 1942). The ash content was 17.5 % (expressed as Cl⁻, the main anion (Christian & Waltho, 1962)), the protein content was 59.7 %, the carbohydrate content was 7.7 % (as glucose) and the carotenoid content was 15.1 % (by difference). This gives molar ratios of protein (from average molecular weight of amino acids in Table 1): carbohydrate:carotenoid (as bacterioruberin) as 26.2:2.0:0.95. The amino acid composition of the complex is shown in Table 1. A small NH₃ peak was recorded and this suggests that little of the glutamic and/or aspartic acid is present as amide. Also, small unidentified peaks were located between Glu-Gly, Met-Ile, Phe-NH₃ and Lys-His.

Table 1. *Amino acid composition of the complex**

Aspartic acid	13.3	Isoleucine	5.6
Threonine	4.1	Leucine	8.9
Serine	2.7	Tyrosine	2.6
Glutamic acid	14.0	Phenylalanine	3.4
Glycine	13.0	Lysine	2.3
Alanine	11.1	Histidine	2.6
Valine	9.1	Ornithine + arginine	5.7
Methionine	1.6		

*Composition is expressed in moles per cent of amino acid.

When stained with anisidine-phthalate chromatograms of the hydrolysed complex showed one spot which co-chromatographed with glucose and another of low *R_F* value which was also ninhydrin-positive and probably a glycopeptide.

DISCUSSION

The extracted complex of carotenoid, glucose and peptide is a monodisperse system of molecules of approximately 9000 molecular weight. In the complex, glucose is bound to peptide and glycosidically to carotenoid since unhydrolysed material is anisidine phthalate negative. The amino acid analysis showed a wider variety of amino acids with less exact molar ratios compared to the carotenoid-glucopeptide of *Sarcina flava* (Thirkell & Hunter, 1969). This bound carotenoid represents one form in which pigment exists in the membrane where all such pigment is localized. Because

none of the chemical or enzymic methods applied (e.g. glycosidases or proteases) liberated material into organic solvents without degradation of the carotenoid, the latter was estimated by difference. An analogy with the carotenoid-glucoprotein from *S. flava* would suggest that bacterioruberin (the most polar free carotenoid) is involved in this complex. A high ash content for the desalted material was recorded. Since many enzymes in this bacterium are salt-dependent (Larsen, 1967), the membrane proteins may also have salt intimately associated with them, eg. as Na^+ and/or K^+ counterions associated with the aspartic and/or glutamic acid residues in the peptide.

D. T. is grateful to the Science Research Council for financial support, and M. I. S. H. is in receipt of an S.R.C. Research Studentship. We thank Dr Serafini-Fracassini for assistance with the amino acid analysis.

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