determine the average molar proportions of the carbohydrates attached to all the glycopeptides. The existence of a D-mannose-containing glycopeptide is in agreement with previous observations that small amounts of D-mannose were attached to the glycopeptides released by rennin action on whole milk (Sinkinson & Wheelock, 1970a).

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Carbohydrates of Bovine α -Lactalbumin Preparations

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Recently there has been renewed interest in the protein α -lactalbumin, because of the discovery of its role as a component of lactose synthetase (Brodbeck, Denton, Tanahashi & Ebner, 1967).

Crystalline α -lactalbumin prepared by conventional methods has been found to consist of the major protein, present in the greater proportion, and a smaller amount of a second component. This secondary protein has the same amino acid composition as the major protein and has been reported to contain 1% of hexosamine (Gordon, Aschaffenburg, Sen & Ghosh, 1968). Apart from this there have been no reports of carbohydrates being present in α -lactalbumin (Ebner & Brodbeck, 1968).

α -Lactalbumin was prepared by ammonium sulphate fractionation of the 3%-(w/v)-trichloroacetic acid precipitate of whole milk (Aschaffenburg, 1968) and by ammonium sulphate fractionation of whole milk followed by separation on Sephadex G-100. Crystalline preparations were also purchased from Koch-Light Laboratories Ltd. (Colnbrook, Bucks., U.K.) and from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.).

Polyacrylamide-gel disc electrophoresis of these preparations showed only the main protein band

and a faster-moving band due to the minor component.

G.l.c. of the trimethylsilyl derivatives of the methyl glycosides released on methanolysis (Clamp, Dawson & Hough, 1967; Sinkinson & Wheelock, 1970) showed that all these preparations contained D-mannose, D-galactose, 2-acetamido-2-deoxy-D-galactose and 2-acetamido-2-deoxy-D-glucose. The total concentration of carbohydrates varied from 0.25 to 1.5%. Assuming that the carbohydrates are attached to the α -lactalbumin, then there is considerably less than 1 mol of each carbohydrate/mol of protein. If all the carbohydrates are associated together in one moiety then this moiety must be present in only a proportion of α -lactalbumin molecules. As hexosamine has already been detected in the minor component it is possible that all the carbohydrates are attached to it. This would provide an explanation for the difference in properties between the major protein and the minor component.

The presence of carbohydrates in α -lactalbumin may have an effect on its role in lactose synthesis.

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The Aggregation Properties of C-Phycocyanin

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Phycocyanin from several algal sources has been examined over a wide range of concentrations (0.005–4.0 mg/ml). Osmotic-pressure, sedimentation-velocity, gel-filtration, disc-electrophoresis and immunodiffusion measurements all indicate that phycocyanin is a highly aggregated protein (Scott & Berns, 1965; Berns, 1967, 1970; Kao & Berns, 1968). Large aggregates exist at both extremely low and high concentrations (Lee & Berns, 1968a,b). Measurements with an analytical ultracentrifuge, equipped with a monochromator and photoelectric

scanner unit, have been performed with 620, 350 and 280 nm light. At all wavelengths sedimentation-velocity measurements gave identical results. Because of the magnitude of the extinction coefficient of the 620 nm absorption band of phycocyanin, measurements could be made at concentrations as low as 0.005 mg/ml. The monomer is not the only species present above concentrations of 0.01 mg/ml. It is possible to stimulate disaggregation to monomer only by employing a pH of 3.9. Band sedimentation and conventional sedimentation-velocity measurements indicate that hexamer is the first aggregate formed. The aggregation process is analogous to a micellar process. The behaviour is consistent with a structural role for phycocyanin in addition to its known function as an energy-transfer pigment. Fluorescence measurements also indicate that the largest aggregates have the highest fluorescence efficiency (Lee & Berns, 1968a). The aggregation process in phycocyanin may be a prototype for membrane-forming systems.

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Structure of Phycocyanobilin

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Phycocyanobilin, the prosthetic group of the algal biliprotein C-phycocyanin, is a linear tetrapyrrolic compound. An extensive study by Ó hEocha (1963) revealed that three different phycobilins can be obtained, depending on the conditions of the hydrolysis of the protein. The three different phycobilins were designated phycobilins 630, 655 and 608. Phycobilin 630 is considered to be the native form (hence the 'real' phycocyanobilin), whereas phycobilins 655 and 608 are derivatives obtained after prolonged treatment with concentrated acid. In view of its u.v. spectrum phycocyanobilin should be structurally intermediate between mesobiliverdin and mesobiliviolin.

A structure for phycocyanobilin has been proposed based on the information obtained from mass and n.m.r. spectra (Cole, Chapman & Siegelman, 1967, 1968; Crespi, Boucher, Norman, Katz & Dougherty, 1967; Crespi, Smith & Katz, 1968).

According to these workers the molecule has a molecular weight of 586, or more precisely a molecular weight of 614 for its dimethyl ester.

Our results (B. L. Schram & H. H. Kroes, unpublished work) indicate that the acid form of phycocyanobilin has a molecular weight of 588 and that the dimethyl ester has a molecular weight of 614 (2 less than expected for a dimethyl ester of a dibasic acid with molecular weight 588).

A close look at the mass spectrum of the acid reveals several properties inconsistent with the structure previously proposed. We therefore present a new structure for phycocyanobilin that has two hydrogen atoms more and that has an internal hydrogen bond.

The new structure can better explain the chemical properties of phycocyanobilin. In the first place, breaking of the internal hydrogen bond, e.g. by esterification or treatment with concentrated acid, causes the molecule to be converted into a more stable dehydrogenated form. The structure given by Cole *et al.* (1967, 1968) will be that of phycobilin 655, since it is the stable form of the ester. It also explains the irreversibility of the transition of phycobilin 630 into phycobilin 655. The n.m.r. spectra are obtained with a solution of phycocyanobilin in trifluoroacetic acid or in deuterated pyridine; the molecule is probably converted readily into its dehydrogenated form in these solvents and the two extra hydrogen atoms will not be observed.

Phycobilin 608 has a violinoid structure and is isomeric with phycobilin 655. A tentative structure will be given, based on an analysis of the electronegative properties of the individual pyrrole rings in phycobilin 655.

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Some Aspects of the Localization of Phycobiliproteins in the Rhodophyceae and the Cyanophyceae

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The phycobiliproteins observed in the Rhodophyceae appear in general in the form of globules (*Porphyridium cruentum*, *Rhodomela subfusca*, *Antithamnion glanduliferum*) but sometimes in the form