Algal Biliproteins

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Biliproteins are chromoproteins that contain bilin prosthetic groups. Plant biliproteins have well-defined and important functions and comprise the photosynthetically active phycoerythrins and phycocyanins of red, blue-green and cryptomonad algae and the photoperiodic regulator, phytochrome, which is widely distributed in the plant kingdom. The difficulty of purifying phytochrome in sufficient quantities for detailed structural work has focused much attention on the apparently closely related algal biliproteins, most of which have been obtained pure in gram quantities from wild macroscopic algae (see Ó Carra, 1965) or from large-scale cultures of micro-algae (cf. Siegelman, Chapman & Cole, 1968).

The phycobilin prosthetic groups of these algal biliproteins, the red phycoerythrobilin and the blue phycocyanobilin, for long proved experimentally intractable owing to their lability and their strong covalent attachments to the apoproteins. More recently, the development of a mild method for releasing these chromophores in good yield (Ó Carra & Ó hEocha, 1966) enabled the successful application of standard physical techniques to their structural elucidation (e.g. Cole, Chapman & Siegelman, 1967; Crespi, Smith & Katz, 1968), and the development of flexible micro-scale degradation techniques led to completely independent elucidations of the structures (Rüdiger, Ó Carra & Ó hEocha, 1967). The structures proposed for phycoerythrobilin and phycocyanobilin are isomeric, and their most unusual feature is an ethylidene side chain on ring A, which has since been detected also in one form of phytochrome (Rüdiger & Correll, 1969).

The light-induced spectral shifts of phytochrome bear a remarkable resemblance to base-induced spectral shifts of the phycobilins that appear to involve the ethylidene grouping (Rüdiger & Ó Carra, 1969). The structural basis of these shifts were discussed.

Rüdiger & Ó Carra (1969) showed that the phycobilins are linked to the apoproteins through a propionic acid side chain and also through ring A. In R-phycoerythrin these linkages appear to be an ester bond to a seryl residue and a labile bond between the lactam grouping of ring A and the γ -carboxyl group of a glutamyl residue (Killilea & Ó Carra, 1968). Alternative proposals, involving linkage through the ethylidene grouping (see Crespi & Smith, 1970), are not consistent with our observations.

The presence in R-phycoerythrin of a urobilinoid prosthetic group in addition to phycoerythrobilin (Ó hEocha & Ó Carra, 1961; Ó Carra, Ó hEocha &

Carroll, 1964) has been questioned by Chapman, Cole & Siegelman (1968), but in this laboratory we have cleanly separated out this urobilin as a chromopeptide from tryptic digests of the biliprotein. The central portion of the prosthetic group (named 'phycourobilin' by Ó Carra *et al.* 1964) is of the classic bilene *b* type, but degradation and isomerization studies reveal the presence of an ethylidene grouping on ring A and the same two linkages to the protein as are characteristic of the other phycobilins. However, the urobilin is additionally linked to the apoprotein through the ethyl/vinyl side chain of ring D, apparently through a thioether bond (S.D. Killilea & P. Ó Carra, unpublished work). It is interesting that this side chain appears to be involved in the chromophore-protein bonding in phytochrome also (Rüdiger & Correll, 1969).

Recently developed analytical techniques have enabled the first completely unambiguous demonstration that the phycobilins are entirely of the IX α configuration (Ó Carra & Colleran, 1970), confirming that they are derived from a porphyrin IX precursor. The elegant demonstration by Troxler, Brown, Lester & White (1970) that phycocyanobilin formation parallels carbon monoxide evolution in *Cyanidium caldarium* suggests that a haem compound is a precursor the phycobilin, by analogy with bilin formation in mammals. Attempts to demonstrate this directly, by incorporation of complete tetrapyrroles, have been hampered by technical difficulties, but the results of some recent direct feeding experiments with *Anacystis nidulans* seem to confirm that haem is a more direct precursor of phycocyanobilin than protoporphyrin (E. Colleran & P. Ó Carra, unpublished work). In view of the specificity of the coupled oxidation of haemoproteins (Ó Carra & Colleran, 1969), it seems likely that the specific formation of the IX α configuration of the phycobilins is brought about by specific α -methine oxidation of haem in a haemoprotein with a binding site similar to that of myoglobin.

Recent work on the apoproteins has been chiefly concerned with subunit size and organization in C-phycocyanin, which has been the subject of much controversy. Earlier *N*-terminal studies (Ó Carra, 1965), which indicated a very large polypeptide size, are reproducible, but the results are affected by some peculiar form of masking (Cope, Smith, Crespi & Katz, 1967; P. Ó Carra, unpublished work) and are misleading. More recently, different groups have consistently advocated quite divergent sizes for the basic subunit, chiefly 30 000 (see Berns & Kao, 1969) or 14 000 (see Bloomfield & Jennings, 1969). We find a polypeptide size of about 20 000 by gel filtration in 8M-urea, following the method of Chao & Einstein (1969), and polyacrylamide-gel electrophoresis in dodecyl sulphate solution (by the method of Dunker & Rueckert, 1969) consistently resolved

two phycocyanobilin-containing components with apparent molecular weights of 18500 and 20500. Since the phycocyanin used in these studies could be readily renatured from 8M-urea it seems unlikely that the results could be attributable to proteolytic degradation.

R-phycocyanin is a complex biliprotein containing both phycocyanobilin and phycoerythrobilin (Ó Carra & Ó hEocha, 1965). Polyacrylamide-gel electrophoresis in dodecyl sulphate solution resolved this biliprotein into a blue subunit containing phycocyanobilin as its only phyco bilin and a red subunit containing only phycoerythrobilin. The blue subunit is slightly the smaller of the two, with an apparent molecular weight of 18500, and the red subunit has an apparent molecular weight of 20500.

Similar studies of both R- and B-phycoerythrins indicated a seemingly homogeneous polypeptide size of molecular weight about 19500, in reasonable agreement with estimates based on chemical studies (see Ó hEocha, 1965). On the other hand, C-phycoerythrin was resolved by dodecyl sulphate electrophoresis into two red components with apparent molecular weights of 19700 and 22000 (S. D. Killilea & P. Ó Carra, unpublished work).

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Subcellular Location of Biliproteins in Plants

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Mechanism of Action of Phytochrome

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Light controls morphogenesis in higher plants through the action of the pigment phytochrome (for a review see Mohr, 1969). The problem is to understand the mechanism of phytochrome action at the molecular level. By this term we designate the temporal sequence of elementary steps from light-absorption to the final photoresponses.

Phytochrome is a bluish biliprotein having two forms that are interconvertible by light: P_r , with an absorption maximum in the red at about 660 nm, and P_{fr} , with an absorption maximum in the far-red at about 730 nm. Since the absorption spectra of P_r and P_{fr} overlap throughout the visible range light of any wavelength will establish photostationary states. Measurements *in vivo* of the percentage of P_{fr} at photoequilibrium as a function of wavelength indicate that in the red about 80% of the total phytochrome is present as P_{fr} , whereas in the far-red only a few per cent is present as P_{fr} . P_{fr} is the physiologically active form of the phytochrome system, i.e. the effector molecule. In a dark-grown seedling only P_r can be detected. The photochemical formation of P_{fr} involves an excitation step and a number of dark reactions. P_{fr} is not stable. In the tissue of the mustard seedling, for example, it decays with a half-life of 45 min at 25°C. To maintain a stationary concentration of P_{fr} in a tissue it is necessary to choose a particular photostationary state with a low $[P_{fr}]/[P_{total}]$ ratio. Under the standard far-red light that we use $[P_{total}]$ is virtually constant in the cotyledons of the mustard seedling for more than 8 h. Synthesis of P_r and irreversible decay of P_{fr} compensate to yield a steady state with 2.5% of P_{total} present as P_{fr} (Marmé, 1969; Oelze-Karow, Schopfer & Mohr, 1970).

Phytochrome is a biliprotein. The chromophore is similar to phycocyanobilin, but the covalent bonds between chromophore and protein are different (Rüdiger, 1969). According to Correll, Steers, Towe & Shropshire (1968) the molecular weight of