COLOURATION IN MARINE INVERTEBRATES - A central role for astaxanthin complexes

For more information contact:
Prof. P Zagalsky
Tel: 0207 7221272
e-mail: p.zagalsky@ntworld.com
Prof. J R Helliwell
Tel: 01925 603236
e-mail: j.r.helliwell@dl.ac.uk
Supported by:
Leverhulme Trust &
UK Research Council's Joint
Biology Programme

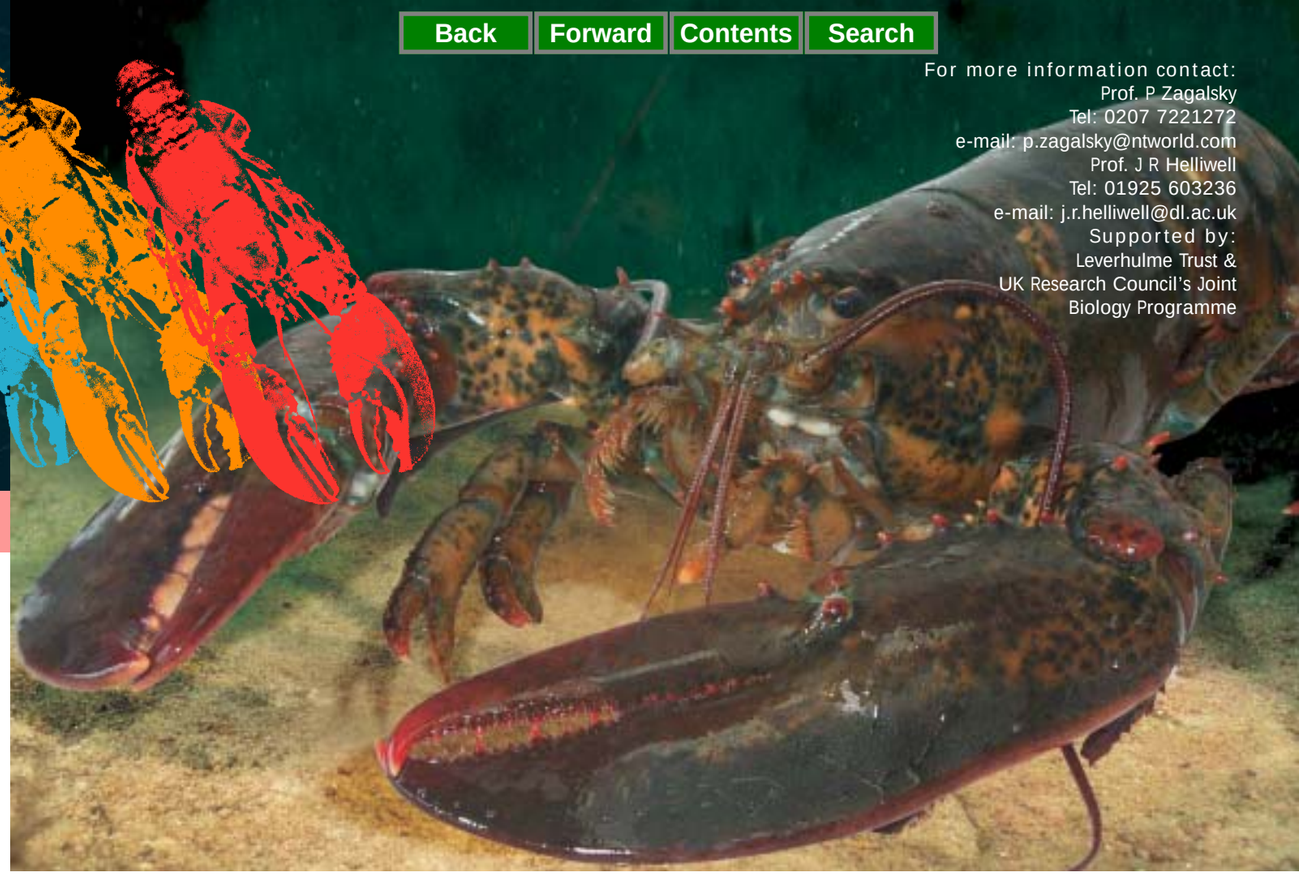
More than 600 carotenoids are found in plants and animals. Carotenoids are the molecules giving rise to the wonderful colours of peppers, tomatoes and other fruits and vegetables. In plants, as chlorophyll/bacteriochlorophyll-protein complexes, carotenoids play roles in photosynthesis such as light-harvesting, photo-protection and singlet oxygen scavenging. In Man, carotenoids exhibit provitamin A activity and act as dietary antioxidants, reducing the risk of cancer and cardiovascular disease, and are widely used commercially as food colourants.

In marine invertebrates, blue and yellow caroteno-proteins are employed for camouflage or in mating rituals as attractants. One particular carotenoid, astaxanthin, is abundant in green and purple/blue caroteno-proteins. Astaxanthin gives marine invertebrates their colour by forming complexes with proteins covering the complete visible absorption spectrum and has a typical absorption maxima (λmax) at 472 nm in hexane. The ability of the protein to alter the spectral properties of astaxanthin is illustrated in three astaxanthin-protein complexes of lobster. The lobster carapace is coloured slate-blue by α-crustacyanin (λmax 632 nm); a yellow protein, crustochrin (λmax 409 nm) is present in the outer carapace layer and lobster eggs are coloured bright green by the storage lipoglycoprotein, ovoverdin (λmax 460 and 640 nm). Crustochrin consists of 20 or so stacked astaxanthin molecules stabilised by protein, which interact by exciton-exciton interaction, thus lowering the λmax. The green colour of ovoverdin results from two carotenoid binding sites, one contributing red the other blue.

The nature of blue/purple astaxanthin-proteins varies in different invertebrates. For example, the blue/purple caroteno-proteins of starfish, Echinodermata, have subunits comparable in size (8-10 kDa) with the light-harvesting complexes of purple bacteria, but with amino acid sequences unrelated to these or any other known proteins. The blue caroteno-protein, linckiacyanin, from the dorsal skin of the calcified starfish, *Linckia laevigata*, is of exceptionally large size (1000 kDa) and contains 100-200 carotenoid molecules per complex. Outside the photosynthetic system, pigment-protein complexes of this nature are rare. Caroteno-proteins from other starfish, e.g. asteriarubin of *Asterias rubens*, have a simpler structure. The chondrophore *Velella velella* (Cnidaria), also known as 'sailor-of-the-sea', has two blue astaxanthin-proteins in its mantle. The largest of these (V620) has a molecular size of approximately 1-2,000 kDa with perhaps some 100 carotenoids bound and subunits of ~ 23 kDa. These have no obvious homology to those of lobster crustacyanin, from which it is far removed on the evolutionary map. In terms of evolution it is indeed amazing that such complex colouration mechanisms develop.

A typical blue/purple astaxanthin-protein, particularly common in Crustacea, is the slate-blue lobster carapace complex, α-crustacyanin. This macromolecular complex has been extensively studied since 1948. It consists of 16 protein subunits of ~20 kDa, each binding an astaxanthin molecule. The subunits are of two types, known as A and C, which combine in pairs with two molecules of astaxanthin to form β-crustacyanin (λmax. 580-590); eight of these are then associated in the native α-crustacyanin. The amino acid sequences of the protein subunits relate to the lipocalin super-family of proteins, which bind small hydrophobic ligands. Crystallographic information is now available for crustacyanin, whereby the structure determination of the A_1 subunit (type C) confirmed its lipocalin nature. The model was subsequently used in molecular replacement to solve the structure of a β-crustacyanin, crystallised under oil over several months by Dr Naomi Chayen, Imperial College, London.
For the first time, it is now clear how the protein alters the absorption properties of the bound astaxanthin. The carotenoid, held rigidly in its centre by protein interactions, is hydrogen-bonded, via histidine and water, respectively, at the 4- and 4'-keto groups. The carotenoid is distorted by end ring flattening to extend the conjugation, and by bending. Thus, it is the way in which the protein subunits clamp the carotenoid molecules that determines their characteristic absorption and colour.

Fig 1 Astaxanthin proteins:
1) Ovorubin, Pomatia canaliculata, 2) Astaxanthin in hexane, 3) lobster crustochrin 4) Lobster lipoglycoprotein ovoverdin, 5) Velella V600, 6) α-crustacyanin, 7) β-crustacyanin.



The 'A1' structure solution was effected in an unusual application of the Daresbury SRS facilities. Stations 7.2 and 9.5 were used to deliver softer and longer X-rays at ~2.0 Å, to enhance the anomalous signal of the inherent S atoms in the protein, and of Xe atoms infused under pressure into the crystal. The ability to measure high-accuracy data with multiple redundancy measurements, made all the difference. Although the structure is very similar to many other lipocalins, molecular replacement methods had not been successful with any model, over many years, including a homology model predicted on the basis of the sequence. The 'A1' structure was refined at very high resolution using data recorded to 1.4 Å, also recorded on station 9.6 at the SRS.

The longer wavelength technique is being used at other laboratories across the world. At the same time, a group at the ESRF, Grenoble, used X-rays at wavelengths of 1.78 Å to solve the structure of the C1 subunit (type C) of crustacyanin, and groups at Elettra, NSLS and Hamburg synchrotrons are exploring yet longer wavelengths. To our knowledge this is the first unknown protein structure solved with softer X-rays.

The use of the A1 model to solve the structure of the A1/A3 β-crustacyanin yielded the first structure of a type A subunit (A3). Despite the low sequence identity the two protein folds are identical. More importantly, we now have a first glimpse of the 'bathochromic' mechanism of how a shift in the absorption of the carotenoid from 480 nm to 590 nm brings about the change in colour from the orange of the free pigment, to the blue of the natural β-crustacyanin. Further work is needed to complete the explanation of the further absorption shift to 632 nm in the native α-crustacyanin. Even more effort is required to compare the lobster system with other invertebrate species. Overall, one can say that the well known phenomenon of cooked lobster changing to orange red is now being understood at the molecular level. In addition, astaxanthin is one of the most potent and bio-active biological antioxidants found in nature and is a popular molecule of choice for the biotechnology and pharmaceutical industry.

GENERAL REFERENCE:

M Cianci, PJ Rizkallah, A Olczak, J Raftery, NE Chayen, PF Zagalsky and JR Helliwell (2001). Structure of Lobster Apocrustacyanin A1 Using Softer X-rays.
Acta Cryst. Vol D57, pp 1219-1229.

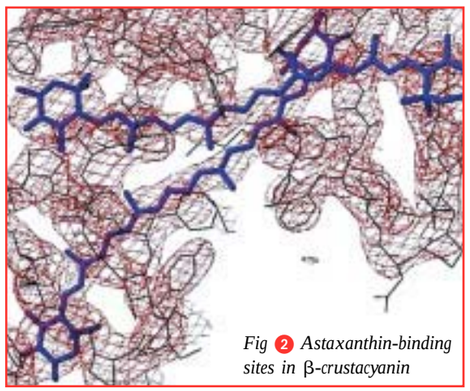


Fig 2 Astaxanthin-binding sites in β-crustacyanin

Fig 3 The purple/blue astaxanthin protein, crustacyanin, confers on the lobster carapace its characteristic colouration (a). The actual colour is a result of the interaction of the red/orange carotenoid astaxanthin with crustacyanin. When the lobster is cooked (b), crustacyanin is denatured, releasing the pigment, which regains its red-orange colour. The structure of β-crustacyanin explains a large part of the spectral shift in the absorption of the pigment when bound.