The chemical basis of the bathochromic shift in the lobster carapace; crystal structures of unbound carotenoids and electron microscopy of α-crustacyanin

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Lobsters have a distinctive blue/black coloration which changes to red on cooking. The colour of the lobster arises from the protein molecule crustacyanin, which has two specifically bound molecules of the red chromophore, astaxanthin (AXT). The crystal structure of β-crustacyanin [1] revealed several candidate colour tuning parameters, which might cause the large bathochromic shift of 150 nm between the free AXT and the protein bound form . A structure study of α-crustacyanin, comprised of 8 β-crustacyanins [2], using electron microscopy is underway, including, so far, a uranyl acetate negative stain structure of the American lobster α-crustacyanin, mainly as a prepeartion for a cryoEM study. Our recent crystallography work has yielded an ensemble of non-protein bound carotenoid crystal structures, which allow some of these key parameters, thought to influence the colour tuning of astaxanthin in crustacyanin, to be varied [3]. The crystal structures of the unbound carotenoids, astaxanthin, canthaxanthin, (3*R*,3*'S)*- zeaxanthin [3], 7,8-didehydroastaxanthin and 7,8,7',8'-tetradehydroastaxanthin are compared with each other and the protein bound astaxanthin molecule in β-crustacyanin. In addition, most recently, s-*cis* and s-*trans* isomers of an astaxanthin ester have been crystallised. The conformations of all these molecules vary, in particular, with the angle of twist of the end rings out of the plane of the polyene chain. Most of the carotenoids, including free AXT, crystallise in the s-cis conformation with the end rings bent out of the plane of the polyene chain by angles of 40 to 50°, but for (3*R*,3*'S)*- zeaxanthin this angle is 74.9(4) ° and for 7,8-didehydroastaxanthin, the end ring torsion angles are 4.8(1) and 14.4(1)°. At the other extreme, the 6-s-trans ester of AXT and 7,8,7',8'-tetradehydroastaxanthin are *s-trans* with the end rings coplanar with the polyene chain, and therefore their conformations are much more similar to that found in the protein bound astaxanthin molecules in β-crustacyanin. Thus a reasonable ensemble of the possible end ring conformations has been determined. The packing of the molecules is also quite different in each case involving a variety of both strong and weak hydrogen bonding as well π-π stacking interactions. However, it is highly significant that despite the differences in end ring conformations and packing arrangements, the colour of each of the crystals remains red, and not the blue of crustacyanin. This leads to the conclusion that one of the other proposed colour tuning mechanisms must be at work in the bathochromic shift seen in crustacyanin, namely, increased polarisation of the polyene chain caused by interactions of the keto oxygens with the protein and/or strong exciton coupling. This latter is a strong possibility for the extra 50nm bathochromic shift in the α-crustacyanin compared with β-crustacyanin. Thus the search for how to mimic the lobster bathochromic colour shift effect continues! Some of the crystals of unbound carotenoids that we seek structures for have proved to be too small for the SRS 9.8 intensity and focal spot size, lycopene is one example, and will be sought with higher brilliance X-ray beam intensities.

[1] M Cianci, P J Rizkallah, A Olczak, J Raftery, N E Chayen, P F Zagalsky and J R Helliwell, *PNAS*, 2002 USA 99, 9795-9800.[2] N Chayen, M Cianci, J G Grossmann, J Habash, J R

Helliwell, G A Nneji, J Raftery, P J Rizkallah & P F Zagalsky Acta Cryst 2003, D59, 2072-2082. [3] G. Bartalucci, J. Coppin, G. Hall, J. R. Helliwell, M. Helliwell, S. Liaaen-Jensen, S. Fisher, *Acta Crystallographica B*63, 2007, 328-337 and manuscript in preparation.

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