MS16 P12

Interaction of Hydrophilic Polymers with Proteins

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Non-covalent interactions between proteins and hydrophilic polymers are widely used in design of drugs with an advanced performance, in food production and in cosmetics. In design of protein based drugs, the polymer chain is usually covalently linked to the protein by a cleavable linker. Long polymer chain winds on the protein surface and provides protection against undesirable effects. For example, PEGylation was proved to provide protection against proteolytic degradation, to decrease toxicity, to enhance permeability of drug through membranes, to improve drug delivery into tumor cells, to decrease inflammatory reactions, to reduce the clearance rate of the drug and to prolong the time of its circulation in body (e.g.[1]).

X-ray structure determination of pure crystalline polymer samples is usually of low accuracy and reliability because of a low structural order in the polymer crystals [2]. The inconvenience caused namely by high flexibility and polydispersity of polymer samples disappears when the polymer molecule is fixed in a regular three-dimensional scaffold of suitable protein crystals with high solvent content. In spite of the fact that we can expect more binding modes of the same polymer and that the polymer chain can be often expected in multiple conformation, the observed segments of polymer chains adhering to the protein surface are clearly visible in the electron density maps as a rule.

The paper summarizes all observed interaction types of polymers of polyether type with two-dimensional patterns of charged and ionizable side chains and the main chain amines at protein surface. The lengths of continuous polymer segments experimentally observed on protein surface are usually about 6 monomers and the longest continuous segment observed was 12 monomers long. The typical experimentally found interaction types were sorted according to the dominating interactions and by the frequency of their incidence. The binding affinity of the individual interaction types was also assessed by interaction energy calculations.

Among variety of observed binding motives, there is one interaction type deserving a special attention - the encapsulation of a dissolved cation by polyether chain followed by binding of the whole polymer complex to the protein surface by hydrophobic interactions (e.g. PDB code 1OBF).

The predominant and strongest binding motives observed are: (a) polymer loop forming multiple charge-supported hydrogen bonds to positively charged side chains (Arg, Lys, His) and

(b) unfolded polymer chain passing through long grooves in protein surface rich for H-bond donors (Asn, Gln, Asp, Glu, Tyr, Ser, Tre, and main chain NH groups) [3].

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MS16 P13

Origin of the colour of lobsters; crystal structures of unbound carotenoids

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Lobsters have a distinctive blue/black coloration which changes to red on cooking. The protein crystal structure of β-crustacyanin with two specifically bound astaxanthins [1] revealed several candidate colour tuning parameters. Our recent work has yielded an ensemble of non-protein bound carotenoid crystal structures, which allow several key parameters, thought to influence the colour tuning of the bathochromic shift of astaxanthin in crustacyanin, to be varied. The crystal structures of the unbound carotenoids, synthetic astaxanthin (3*S,*3*'S;* 3*R,*3*'R*) in a 1:2:1 ratio), canthaxanthin, 7,8-didehydroastaxanthin (found in starfish) and (3*R,*3*'S)*-zeaxanthin are compared with each other and the protein bound astaxanthin molecule in the lobster carapace carotenoprotein, β-crustacyanin. Three new crystal forms of astaxanthin have been obtained, using different crystallisation conditions, including a chloroform solvate, a pyridine solvate and an unsolvated form. In each carotenoid crystal structure, the carotenoid molecules adopt the 6-s-*cis* conformation; the end rings are bent out of the plane of the polyene chain by angles of -40 to 50° for all the structures except for zeaxanthin, where this angle is -74.9(3)° and for 7,8-didehydroastaxanthin, where the angles are 177(3) and 129(3)° . The packing of the molecules is quite different in each case involving hydrogen bonding and π-π stacking interactions. Theses studies are described in [2]. In addition, most recently, s-*cis* and s-*trans* isomers of an astaxanthin ester have been crystallised, where the s-*trans* isomer has a similar conformation to that found in the protein bound astaxanthin molecules in β-crustacyanin. These crystal structures are compared with one another, as well as with the astaxanthin molecules in the 3.2Å protein crystal structure of β-crustacyanin. The fact that the colour of each of the crystals remains red, and not blue, is therefore especially significant. The search for how to be able to tune the lobster bathochromic colour shift effect continues!

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