The 3.2 Å resolution X-ray structure of Panulirus interruptus hemocyanin

Volbeda, Anne

Summary

Hemocyanin is an oxygen carrying protein which occurs in a large number of invertebrates. Two classes are distinguished, the arthropodan hemocyanins and the molluscan hemocyanins, which have a very different structure. The arthropod whose hemocyanin is discribed in this thesis is the spiny lobster *Panulirus interruptus*. As all hemocyanins it contains a dinuclear copper site, where the oxygen is bound. The structure of *Panulirus interruptus* hemocyanin, which is a hexamer, has been solved a few years ago. The main part of the research which resulted in this thesis was devoted to crystallographic refinement of this structure, which until now is still the only solved hemocyanin crystal structure.

The oxidation state of the copper ions of the oxygen binding site is I in the colourless deoxyhemocyanin and II in the blue oxyhemocyanin. Also in the colourless met-form of hemocyanin, which is not able to carry oxygen, the dinuclear copper site consists of two Cu(II)-ions. Cu(I) and Cu(II) can be distinguished by X-ray absorption spectroscopy. In chapter 2 the results of X-ray absorption measurements on hemocyanin crystals are presented. The most important result is that the crystals used for the X-ray structure determination practically contain only Cu(I)-ions. This shows that the structure of deoxy-hemocyanin has been determined.

After the building of a complete atomic model of hemocyanin, the next step in the structure determination was crystallographic refinement. This is described in chapter 3. Refinement was performed by means of a least squares procedure in which shifts in atomic coordinates are calculated in order to minimize the discrepancy between measured structure factors and structure factors which are calculated from all atomic positions in the model. As the *Panulirus interruptus* hemocyanin hexamer consists of more than 32,000 non-hydrogen atoms, practical refinement was only possible with a supercomputer. After many cycles of refinement and much manual intervention the crystallographic R-factor of the final model became 20.1 %. Used were about 59,000 reflections between 8.0 and 3.2 Å resolution. The mean coordinate error of the final model is probably less than 0.35 Å.

Structure analysis shows that the hexamer is best described as a trimer of dimers. Three domains can be distinguished in each subunit. The second domain occupies a central position, whereas domain 1 and domain 3 are located more on the outside of the hexamer. Most of the intersubunit contacts are mediated by residues of domain 2. This domain also contains the oxygen binding dinuclear copper site. Three large internal cavities probably facilitate fast binding of oxygen.

The dinuclear copper site is surrounded by a large number of hydrophobic residues. Both copper ions are bound by three histidine ligands, two of which are at the short distance of about 2.0 Å and the third at a relatively large distance of about 2.6 Å. The two weakly bound histidines are located at opposite sides of the plane in which the two copper-ions and the four tightly bound histidines are approximately situated. A geometric analysis and a comparison with the structure of model Cu(I)-compounds suggests the presence of en extra copper bridging ligand, which might be OH^-. However, no bridging ligand is visible in the electron density map, but this might be due to the relatively low resolution of 3.2 Å.

The copper-liganding histidines are provided by two α-helix pairs. Two histidines come from the first helix of each pair, in which they are separated by three residues or

one helix-turn. The third histidine is provided by a second helix. There exists a pseudo-twofold symmetry, which relates the two copper binding helix pairs, as discussed in chapter 4. The pseudo symmetry involves 31 residues in the two helix pairs, 8 of which are identical. After optimal superposition the corresponding 31 C^{α}-atoms show a r.m.s. deviation of less than 1.5 Å. This low r.m.s. deviation, the relatively large number of identical residues and the observation that the pseudo symmetry is almost twofold seem to point that the oxygen binding site evolved a long time ago by dimerisation of a copper binding helix pair, followed by gene duplication, gene fusion and divergence of sequences.

The evolutionary scenario described above is supporded by the recent discovery of a striking homology between the Cu(B) binding helix pair of arthropodan hemocyanin and a fragment of the aminoacid sequence of a molluscan hemocyanin. No part of this molluscan hemocyanin resembles the Cu(A) binding binding helix pair. It appears that arthropodan and molluscan hemocyanins, in spite of their present rather different structure, have a common ancestor: a copper binding helix pair.

Two other classes of oxygen carrying proteins found in nature are hemerythrin and hemoglobin. Also in these rather different proteins helix pairs are important for oxygen binding, two in hemerythrin and one in hemoglobin, but here they surround iron instead of copper. These helix pairs show striking similarities (see chapter 4) when compared to each other and also when compared to hemocyanin. Therefore it may be speculated that a metal binding helix pair is the common ancestor of the known four classes of oxygen carrying proteins: arthropodan hemocyanin, molluscan hemocyanin, hemerythrin and hemoglobin.

The hemocyanin structure described in this thesis may be much further analyzed. For example, it may be used to construct detailed models of multihexameric hemocyanins. It would be very interesting to have the structure of an oxygenated hemocyanin, in order to compare this with the structure of *Panulirus interruptus* deoxy-hemocyanin. Possibly even more interesting would be the structure determination of a molluscan hemocyanin, after which there would be at least one crystal structure of each of the four known classes of oxygen carrying proteins. Much research is still to be done with respect to the structure-function relation in hemocyanin. Hopefully a lot of progress will be made yet in Groningen with exploring this very interesting protein.