C03B44XU-7402-N BL44XU

X-ray structure analysis of anthocyanin pigment complexes

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An anthocyanin complex from a flower pigment was crystallized for X-ray structure analysis. The native comlpex contains some kinds of metals. Since the native crystal has needle like shapes and not suitable for X-ray diffraction, we prepared several metal-substituted crystals.

We have already been analyzed several metal-substituted crystals. However, we could not find one of the metals which is assumed to play an important role in coloring mechanism of this pigment. In order to determine the position of the missing metal, we substituted the missing metal for several other metals. This time, one of these crystals was chosen for X-ray measurement.

The data collection was made at the beam line BL44XU for macromolecular assemblies. The data were collected by using DIP2060 area detector up to 1.05 angstrom resolution. X-ray wavelength of 0.7 angstrom was used. The space group of the metal-substituted complex is P 63 2 2 with cell parameters of a=32.27 and c=28.50 angstrom.

Since the crystal is isomorphous to several other crystals. We started the analysis from another metal-substituted structure which has been solved by the authors. The refinement was carried out with SHELX97.

Including this crystals, four kind of crystals have already been solved, in which the missing metal is substituted for other metals. Nevertheless, the metal in question is still not found. Instead, when the kind of substitutional metal has changed, it has turned out that the electron density of another metal varies. The value of the density is approximately an average with the density of the substitutional metal and that of the metal which is assumed to occupy this site.

Our conclusion is that the missing metal shares one site with the other metal at the ratio of 1:1. With this assumption taking into account, the refinement has carried out. The final R-factor has been 6.55 % with 4227 all independent reflections and 6.40% with 4122 reflections of F > 4sigma(F).

C03A44XU-7412-N BL44XU

Crystal Structure of human T-protein of glycine cleavage system

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T-protein, a component of the glycine cleavage system, is a H_4folate-dependent enzyme that catalyzes the formation of ammonia and 5,10-CH_2-H_4folate from the aminomethyl moiety of glycine. We determined the crystal structure of human T-protein (HT) in its native form and complexed form with the substrate analogue, 5-CH_3-H_4folate, at 2.0 Å and 2.7 Å resolution, respectively, using experimental phases obtained by a single wavelength anomalous diffraction (SAD) approach. It comprises three domains arranged in a ring-like structure and 5-CH_3-H_4folate occupies the central cavity of the ring (Fig. 1). The pterin group of the ligand is bound by hydrogen bonds with Glu^{204}, Arg^{233}, and oxygen backbone atom of Val^{115}. The glutamyl group of 5-CH_3-H4folate interacts with Tyr^{371} and Lue^{88} (Fig. 2), confirming our previous evidence that the vicinities of these residues of *E.coli* T-protein were involved in cross-linking with the glutamyl group of 5,10-CH_2-H_4folate. Furthermore, Gly^{241}, Asp^{248}, and Arg^{292}, whose mutation lead to the onset of non-ketotic hyperglycinaemia, are located around the central cavity, supporting the space of ligand insertion .by hydrogen-bonding each other. Kinetic analysis of wild-type and mutant human T-proteins, G241D and R292H which identified in the patients, showed a remarkable reduction in the affinity for folate substrates consisting with the crystallographic result. T-protein shows strong structural similarity with C-terminal half of dimethylglycine oxidase from *Arthrobacter globiformis*.

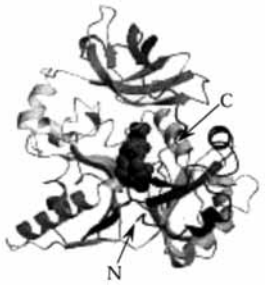

Fig. 1. The overall structure of HT with 5-CH_3-H_4folate rendered in CPK sphere. N- and C-terminus are labeled.

Fig. 2. Stereo view of the HT active site. The *Fo-Fc* electron density map of the ligand is contoured at 2σ.