Experiments on the Diffusion of Dyes and Ions into Protein Crystals

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Experiments have been carried out on 15 different protein crystals, most in the range of 0.5 to 1.5 mm dimensions, to measure the rates of diffusion of dye molecules into crystal interiors. Measurements have also been made of the diffusion of dyes through protein solutions of 50 mg/ml to 400 mg/ml concentration for comparison. We found in the course of our study that for most protein crystals, once saturated with dye (as indicated by their rich colors), the dye is retained in the crystals for at least six months, perhaps indefinitely, after the crystals are submerged in large volumes of clear, dye free mother liquor. This indicates strong association of the dyes with the interior of the crystals. Dialysis experiments further indicate a strong association between the dyes and the protein molecules even in solution. X-ray diffraction experiments on over 25 dye-saturated lysozyme, thaumatin and trypsin crystals, however, failed to reveal (with a few noteworthy exceptions) any difference electron density indicative of ordered binding. This raises the question of how high affinity between proteins and small molecules can arise from completely disordered interactions.

Some of the dyes we use are pH sensitive (pH indicator dyes) and change color as a function of H+ concentration. We exploited the color change of numerous dye-saturated crystals to measure the rates of H+ movement into and out of crystals as the pH of the mother liquor was changed. Some other dyes are sensitive to reduction by, among others, bisulfite or dithionite. Again, color changes within the dye-saturated crystals were used to measure the rates of flow of reductants into the crystals and the rates of subsequent reoxidation of the crystal bound dyes by ambient oxygen. We were further able to saturate protein crystals simultaneously with pH sensitive dyes and redox sensitive dyes and then produce a sequence of color changes in protein crystals by addition of reductants followed by changes in pH of the mother liquor.

Finally, we made the observation that, in general, crystals grown from PEG or other polymers of similar characteristics, unlike those grown from salt, MPD, low ionic strength, etc., can not be stained using any of the more than 30 dyes we investigated. Dyes appear to be barred from entering these crystals. We will discuss possible implications of this observation for crystals grown from PEG, and the mechanism by which PEG promotes crystallization.