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X-ray Crystallography at Extremely Low Temperatures

Chilling protein crystals enables the characterization of transient structural intermediates

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Conventional X-ray crystallography is useful for looking at macromolecular structures that are stable at ambient temperatures. But in enzymic and even in binding processes, intermediate structures are often more informative. By their nature, of course, intermediate structures do not last long enough under normal conditions to obtain X-ray diffraction data. However, by cooling protein samples to within a few tens of Kelvin of absolute zero, the lifetime of the intermediate states can be extended and their structures determined.

It has now become routine for macromolecular crystal structures to be determined at low temperatures, around 90-100 K. This is done by mounting the crystal in a thin fiber loop[1] rather than in the more conventional X-ray capillary, and by bathing the crystal and loop in a cold gas stream derived from the boil-off of liquid nitrogen.

As with studies at room temperature, this procedure yields a time average over all structures present during the X-ray exposure time and a space average over all structures in the crystal. However, key tertiary structural changes that accompany processes such as enzyme catalysis, photocycling, and ligand binding and release are often extremely fast at room temperature, and transient structural intermediates cannot be visualized even by the time-resolved crystallographic techniques presently under development.[2] Under this circumstance, the only recourse may be to work at cryogenic temperatures, in order to slow down these structural processes and prolong the lifetime of the structural intermediates to the point where they can be readily observed.

Two groups[3,4] have recently adopted this approach in studying the photostimulated process of carbon monoxide release (and subsequent rebinding in the dark) from the small heme protein myoglobin, in single crystals. In solution at room temperature, the "germinate" component of the rebinding reaction, in which the carbon monoxide recombines directly with the heme from which it has just been liberated by light, is extremely rapid; spectroscopic studies show that heme structural changes occur on subnanosecond time scales. As very expensive studies, principally by Frauenfelder and colleagues over the past 20 years, have shown,[5] the rebinding reaction and the associated heme and protein structural relaxation are both extremely complicated and exquisitely dependent on temperature.

The lifetimes of spectroscopically detectable intermediates—which presumably also differ in their heme and protein structure—can be prolonged by many orders of magnitude, by working at temperatures between 10-80 K, to the seconds time scale or even longer. Two groups, one[3] working at 40 K using a new cryostat[6] based on the boil-off liquid helium, and the other[4] at 20 K, both succeeded in trapping a normally short-lived intermediate. In this intermediate, the carbon monoxide molecule has been photodissociated from the heme yet remains nearby, and the heme and its surrounding protein have partly[3] or substantially[4] relaxed towards the unliganded, deoxymyoglobin form.

The crystallographic results differ in detail, pointing to a potential difficulty of cryogenic crystallography. Results may depend on fine aspects of the cooling protocol (extremely rapid cooling would "freeze in" the room temperature structural distribution, but in practice this is not achievable, and different parts of the crystal freeze at different rates) and on the exact protocols used to illuminate the crystal, to photo-initiate the reaction and to acquire the X-ray diffraction data (the energy of all visible and X-ray photons absorbed largely appears as heat and may promote structural relaxation). Finally, the task remains to demonstrate that intermediates which are stabilized at cryogenic temperatures indeed are identical to, or differ in verifiable ways from, those at room temperature.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

FIGURE 1. Conventional X-ray crystallography gives a structure of stable myoglobin (featured), whereas using extremely low temperatures can show the protein in action. Picture courtesy of Oxford Molecular, using atomic coordinates from the Protein Data Bank.

The technology to execute such experiments is clearly in place. We now need more experience to assess these questions.

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