Protein crystallography

Catching up with fast changes

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DESPITE its limitations, the technique of millisecond protein crystallography promises to extend the range of biochemical problems that can be studied by X-ray diffraction. The work of J. Hajdu *et al.*, reported on page 178 of this issue, demonstrates the potential of using the static tool of crystallography to gain insight into the workings of a protein as it changes conformation during a reaction.

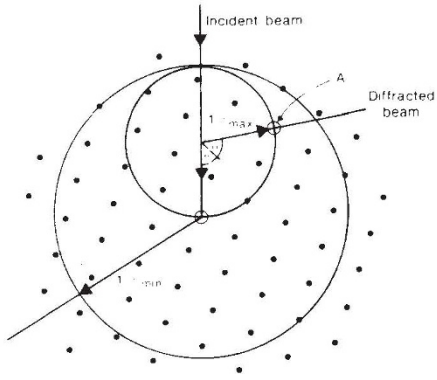
Biochemists have spent much time and energy on the elucidation of enzymatic mechanisms, devising clever methods to identify the reactive groups (those essential to the catalytic process) in a protein. Even cleverer methods can identify intermediates along the reaction pathway. Anomalous results — unexpected spectral changes, for instance — are ascribed to conformational change, the universal catch-all. The protein is attributed with flexibility, some of it caused directly by the chemical process being catalysed.

The advent of protein crystallography, allowing structures and changes in structures to be observed directly, promised a radical enlightenment. Unfortunately, partly because of a misunderstanding of the limitations of crystallography, the hoped-for information has not turned out to be as helpful as was expected. The long time periods (days) required for the collection of protein crystal-diffraction data means that the structures obtained are positional averages, and any short-lived intermediates or structural motions — for example, conformational changes — cannot be observed. Structural information is therefore limited to species that are stable for long times, such as enzyme–product complexes when product inhibition is strong, and any structures that occur in between are lost. Attempts to trap intermediates by, for example, the use of low temperatures, have met with only limited success.

A new approach to this problem recently appeared in the form of molecular engineering. Carefully selected changes in the identity of reactive groups in an enzyme could, in principle, alter the mechanism so as to trap intermediates or provide chemical evidence for the function of a specific group in the mechanism. But careful structural analysis of mutants designed to elucidate specific functional questions either has not been done or has failed to explain the change in reactivity.

Summa sumarum, biochemists have become disillusioned with crystallography, and crystallographers have become impatient with biochemistry. It seems that a new technique is needed to provide structural information on short-lived (the timescale of a biochemical reaction) intermediates along a reaction pathway. Such a technique needs the acquisition of diffraction data on a sub-second timescale. The successful application of such a technique is described in the work of Hajdu *et al.* reported in this issue. The method, which is 75 years old but has never previously been applied to protein crystallography, is called Laue diffraction (Friedrich, W., Knipping, P. & von Laue, M. *Sber. bayer. math. phys. Klasse (Kgl.) Akad. Wiss.* 303–322; Munich, 1912).

Conventional X-ray diffraction uses a single-wavelength X-ray beam. Diffraction from a crystal is observed when Bragg's law is satisfied for any lattice point: $\lambda = 2d \sin\theta$, where θ is the angle of the incident beam to the diffracting plane, d is the interplanar distance, and λ is the wavelength of the incident X-ray beam. Traditionally, diffraction data are collected by varying θ and keeping λ fixed. The number of reflections satisfying the Bragg condition depends on the crystal orientation, and many positions are needed to obtain a complete data set. If a spectrum of wavelengths is used, then a larger number of reflections will satisfy the Bragg condition simultaneously (see figure) and, in favourable cases, a complete data set could be obtained with as little as one exposure. This is the principle of Laue diffraction. If the polychromatic X-ray beam is of very high intensity, such as that produced by a synchrotron, the method has the potential for obtaining a data set on a very short timescale (Moffat, K., Szebenyi, D. & Bilderback, D. *Science* **223**, 1423–1425; 1984). Complications arise because there are harmonic and spatial overlaps limiting the number of reflections that can be observed independently. Spatial overlaps can be resolved by choosing the orientation of the crystal and adjusting the crystal-to-film distance; harmonic overlaps comprise less than 17 per cent of any data set (Cruickshank, D.W.J., Helliwell, J.R. & Moffat, K. *Acta Cryst.*, in the press), so can be ignored.



The spots represent one plane of a lattice of reflections. A reflection is observed if it lies on the circle defined by the wavelength of the original beam. Thus, reflection A lies on the circle defined by $1/\lambda_{max}$ and is observed if the incident beam has wavelength λ_{max}. To observe all reflections, the lattice (the crystal) must be moved into all possible orientations such that the spots (reflections) cross the circle. With a polychromatic incident beam, there is a circle for every $\lambda_{min} < \lambda < \lambda_{max}$ and all reflections between the limits defined by $1/\lambda_{min}$ and $1/\lambda_{max}$ are observed with one orientation of the lattice.

The work described in the paper of Hajdu *et al.* in this issue clearly illustrates the feasibility of this method. The authors monitored the binding of the oligosaccharide maltoheptose to glycogen phosphorylase *b* by taking Laue data sets before, during and after the addition of the ligand. The synchrotron X-ray source at Daresbury in the United Kingdom provided a polychromatic radiation beam 0.2–2.1 Å in wavelength. Each data set required three independent X-ray photographs and took a total of 3 s to collect. Spatial and harmonic overlaps and selection of reliable measurement pairs reduced the unique data set to 25 per cent of the possible unique reflections, but even so, electron-density maps calculated from these data are clear and interpretable, comparable in quality to maps from monochromatic data. The half-saturation binding time for the system analysed by Hajdu *et al.* is about 8 min and so easily falls within the time constraints of the data collection. Maltoheptose can clearly be seen bound to the glycogen storage site of the enzyme.

Even with this breakthrough, a crystalline enzyme system still has to meet certain criteria for the stages of a reaction to be followed using Laue diffraction. Transient intermediates, with half-lives of less than 250 ms, are still not observable, but any accumulating intermediate will be. It will be necessary to choose reactions which can be triggered in such a way that all molecules in a crystal are reacting at the same point along a reaction pathway within the timescale of successive exposures. There are several systems that meet these criteria, for instance, reactions triggered by photochemical means; by the introduction of a metal ion that diffuses rapidly into a crystal (Farber, G.K. *et al. Proc. natn. Acad. Sci. U.S.A.*, in the press); by a temperature jump; or by using mutants to slow down a reaction without altering its mechanism. Complete data sets with a single exposure are possible only if the enzyme crystallizes in a high-symmetry crystal form. Despite these limitations, the Laue method promises new insights into protein mobility and function. □

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