ON THE DETERMINATION OF ENZYME STRUCTURE, FUNCTION, AND MECHANISM

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Contents

Summary

Enzymes are the catalysts of chemical reactions in living cells. They are macromolecules consisting mainly of polypeptide chains formed from amino acids. To understand how enzymes function, their structure must first be known. There is one main technique applied in structural studies of enzymes—crystallography. Enzymes can be crystallized and the crystal structure determined by diffraction of X-rays from the crystal. Recent technical advances in crystallography, as well as better computer programs and faster computers, have made it much more rapid in solving enzyme crystal structures. The accumulating data on enzyme structures—and novel approaches, particularly genome projects and bioinformatics—are expected to increase our understanding of enzyme function and mechanisms in the future.

1. Introduction

Enzymes are proteins that catalyze the chemical reactions needed for the normal behavior of all cells and living organisms. Enzymes, together with other proteins, are needed for all types of functions taking place in and by living cells, for example, the functioning of metabolism, capturing and transforming energy, storing and degradation of substances, reproduction, and defense.

Enzymes are linear polymers of similar building blocks called amino acids (see Figure 1). Amino acids are either obtained from food or synthesized in cells, and polymerized according to the instructions of the genes of the organism. Enzyme molecules fold into three-dimensional structures in order to provide a suitable environment for a particular chemical reaction to occur. The structure formed is such that those amino acids needed as reactants in the chemical reaction come close to each other even though they would be far apart in the linear chain of amino acids (a polypeptide). Each enzyme is specific for one or a very limited amount of compounds, which they bind into their active sites and transform into products of the corresponding chemical reaction. Thus, the enzyme and its substrate—the compound that the enzyme binds—fit together like key and lock. Many enzymes also contain metal ions (or other ligands) embedded inside the folded polypeptide chain. An example of an enzyme molecule structure is given in Figure 1.

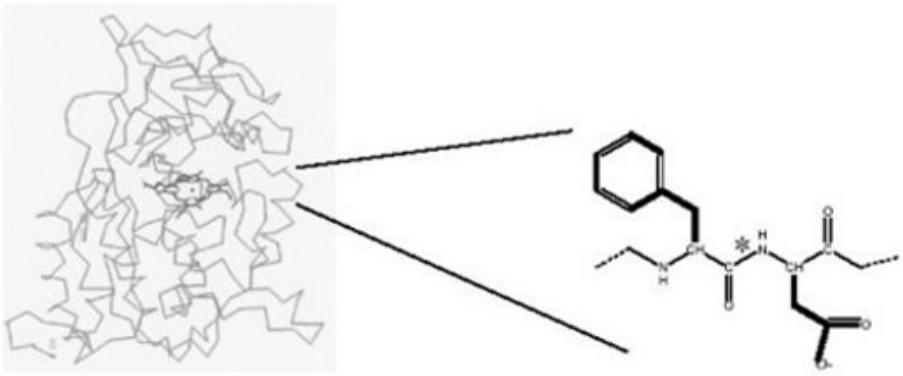

Figure 1. Left: schematic drawing of the structure of the enzyme lignin peroxidase. The polypeptide chain of lignin peroxidase is folded into mainly alpha-helical secondary structures, and it also contains a heme molecule as a cofactor (thicker lines in the middle). Right: magnification of a small portion of the polypeptide chain indicating two amino acids (phenylalanine and aspartic acid) linked by a peptide bond (*). The main chain is running horizontally from left to right, with side chains drawn in bold.

Much of our understanding of the function of living cells depends on knowing the function of enzymes. This in turn is invariably dependent on knowing the structure of the enzyme, but also its cellular location and regulation. For a chemical analysis of an enzyme, however, the most important aspects are knowing the structure of the enzyme at atomic detail and knowing the kinetics, that is, which substrate or substrates the enzyme binds, how fast the chemical reaction takes place, what are the products, and so on.

All atomic detail structures of proteins are collected in a worldwide repository—the Protein Data Bank (PDB). At the time of writing there are 54076 entries in the PDB, of which 28330 (52%) were enzymes. This article explains in principle how to determine the exact, atomic detail structures of enzymes, and how this knowledge can be used in understanding an enzymatic reaction. Additionally, some thoughts are given on how these things are expected to develop in the future.

2. Structure Determination Techniques

There is basically one noteworthy technique for obtaining experimental structural knowledge of enzymes at atomic detail: X-ray crystallography of enzyme crystals. Nuclear magnetic resonance spectroscopy (NMR) of proteins in solution could also apply, but it has certain limitations that render it more suitable for determining structures of proteins other than enzymes (see Section 2.2). The situation is well exemplified by noting that while 83% of all entries in the PDB come from crystallographic work, the corresponding number for enzyme entries is 96%. Therefore, this article focuses mainly on crystallography.

2.1. X-ray Crystallography

2.1.1. General

Crystallography is the science of crystals. Enzymes can be crystallized and the diffraction of radiation from enzyme crystal lattices can be studied and used to determine the positions of atoms of an enzyme just as for any other crystals (e.g., minerals or inorganic salts). However, the particular crystallographic techniques and experimental setups are quite specific for work with enzymes (proteins). Therefore, this branch of crystallography is known as protein crystallography.

2.1.2. Crystallization of Enzymes

Crystals may form when pure chemical substances are subjected to favorable physico-chemical conditions. In terms of enzymes, "pure" means that the experimental sample should contain only one type of enzyme and not be a mixture of different ones. This is usually achieved by cloning the gene coding for the enzyme in a bacterium or other suitable production host cell, letting the bacterium grow and produce large amounts of the enzyme in its cellular machinery, extracting the enzymes from the cell, and separating the enzyme by physico-chemical techniques from other proteins produced concomitantly in the host cell. Several milligrams—sometimes, tens of milligrams—of pure enzyme are usually needed for a structure determination study. In many cases this is a fairly straightforward process nowadays.

Enzymes have bound water molecules as part of their structure. Most waters are situated on the outer surface of enzyme molecules and are essential for maintaining the folded structure of enzymes. If enzymes in solution are subjected to chemicals that, in a chemical sense, compete for water with them, the enzyme molecules may be physically forced to arrange themselves in a crystal lattice and trap the water molecules inside the crystal to stay active. Experimental conditions for enzyme crystallization must be designed so that this is achieved slowly, say, over a few days. Typically, for one single experiment, 2 μg of enzyme is needed and the crystals should reach a size in the order of 0.1 mm (containing in the order of 10^{15} enzyme molecules). A typical setup and some crystals are shown in Figure 2.

The physico-chemical conditions—where a particular enzyme crystallizes—cannot be predicted; instead they must be determined experimentally by trial and error. As

thousands of enzymes have already been crystallized—many of them in several different conditions—certain conditions have been recognized as more successful than others. It has become a general practice—and a very productive one—to first try those frequently successful conditions before conducting a systematic study of alternating pHs, precipitants, temperatures, and other factors that influence the behavior of enzyme molecules in solution. This rather "nonscientific" approach to enzyme crystallization demands, when successful, much less protein, and has therefore made it possible to obtain crystals of enzymes for which only relatively small amounts are available.

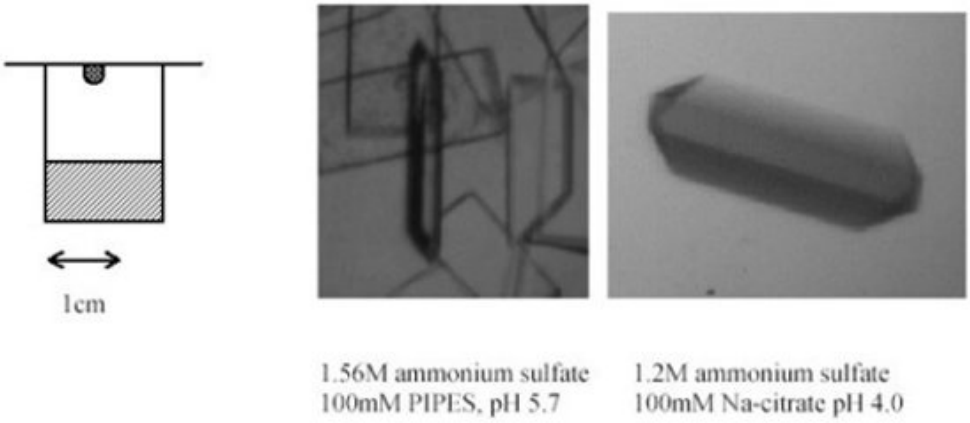

Figure 2. A vapor-diffusion, hanging-drop experiment for enzyme crystallization (on the left). A drop of enzyme solution is placed in a closed container above a solution containing the chemical that induces the crystal formation (precipitant). The experiment is followed periodically from above with a microscope. Enzyme crystals with their crystallization conditions indicated: 3-carboxy-*cis*,*cis*-muconate lactonizing enzyme from *Neurospora crassa* (in the middle) and lignin peroxidase from *Phanerochaete chrysosporium* (on the right). The crystals are approximately 0.5mm long.

2.1.3. X-ray Diffraction Experiments

Judgment of the suitability of enzyme crystals for structure determination is based on their ability to interact with X-rays. The experimental setup (see Figure 3) can be described as follows: a narrow beam of monochromatic X-rays of suitable wavelength is directed to the crystal; X-rays, being electromagnetic waves, either traverse straight through the crystal, in between the enzyme molecules, or hit the electron clouds of the atoms in the enzyme molecules.

In the latter case the wave deviates to some direction away from its original one—in other words, it is diffracted. Molecules arranged side-by-side (in all directions) in a periodic way form a lattice—the crystal—from which the waves diffracted to the same directions accumulate and strengthen each other to produce diffraction maxima that can be recorded by sensitive detectors.

Good crystals are characterized by having high internal order of the molecules (low mosaicity), possessing diffraction maxima at high resolution (preferably, to at least 2.0–

2.5 Å and with reflections visible on the outer edge of the diffraction picture), and having at least a reasonable amount of internal symmetry and suitable unit cell dimensions to compromise with technical challenges of each experiment.

The wavelength of the X-rays used must be in the order of 0.7–1.5 Å to match with the details—the interatomic distances in the enzyme molecule—that one aims to resolve. In addition to X-rays—produced with rotating-anode X-ray tubes—synchrotron radiation is also used.

Synchrotrons are large nuclear physics research facilities producing, among other things, a broad-spectrum electromagnetic radiation from which a desired wavelength can be selected. The advantages of using synchrotron radiation include better quality measurements, a tunable wavelength, and the strength of the radiation, which allows the use of smaller crystals. Disadvantages include traveling with crystals to synchrotrons, which may be located far away, and the tiresome round-the-clock working days necessary in order to use the valuable measurement session most efficiently.

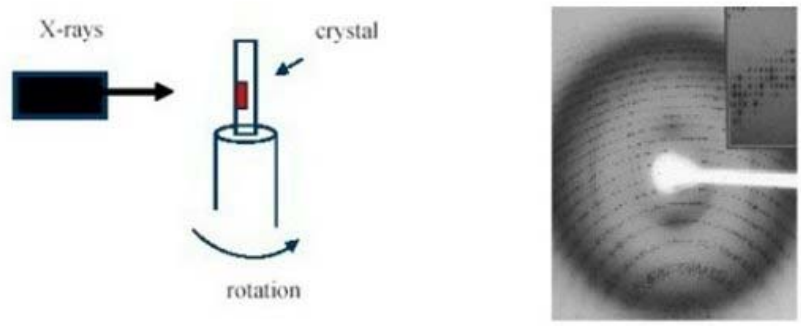

Figure 3. X-ray diffraction experiment (on the left). The crystal is rotated during the experiment in order to record all possible diffraction maxima (data set) obtainable from the crystal; that is, the diffraction picture changes when the incident X-ray beam hits the crystal lattice from an altered angle. On the right is a typical diffraction picture showing a clear periodicity and variation in intensity of the diffraction maxima (reflections). The insert is a magnification of a part of the picture.

Important technical improvements have taken place to automate and speed up the measurements. Fluorescence-based scannable image plates and CCD cameras have totally superseded X-ray films as detectors. While the power of standard laboratory X-ray tubes cannot be greatly enhanced (for technical and economic reasons), the outcoming X-ray beam can be focused to increase the efficiency of the measurements.

Enzyme crystals are almost invariably frozen during the experiment—typically to 100 K. This is achieved by directing a cold stream of nitrogen gas onto the crystal. Freezing makes the crystal tolerant to damage by the radiation and may also contribute to increased data quality. Additionally, freezing may sometimes help in trapping substrates or other molecules that bind to the enzyme to become part of the structure, which is fundamental for structure-function studies.

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Biographical Sketch

Tuomo Glumoff is a biochemist whose research interest is protein science, especially protein structure determination by X-ray diffraction. He obtained an M.Sc. at the University of Turku, Finland in 1987, and a Ph.D. in Biochemistry at the Swiss Federal Institute of Technology, Zürich, Switzerland in 1992. He was a Post-doctoral Fellow at Turku Centre for Biotechnology, 1992–1996, followed by an appointment to a Junior Research Fellowship of the Academy of Finland, 1996–1999. Since 1998 he has been a Senior Lecturer at the University of Oulu, Finland, and Coordinator of the Structural Biology Research Program of the Academy of Finland, 2000–2002.