Structure of an enzyme revealed 80 years after it was crystallized – differential functional behaviour of plant and microbial ureases uncovered

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It is indeed a proud moment for Indian structural biologists, in particular to the Madras University crystallography group who have recently made a contribution of historical significance, by determining the three-dimensional structure of the jack bean urease (JBU). JBU is not only the first enzyme ever to have been crystallized back in 1926, but also one that has demonstrated that enzymes are proteinaceous. James B. Sumner shared the Nobel Prize in chemistry with John Northrop and Wendell Standley in 1946 for this work. Surprisingly, JBU had to wait for more than 80 years for its structural revelation! It may be recalled that it is at the University of Madras, that structural biology research began in this country, under the eminent leadership of G. N. Ramachandran, with the discovery of the triple helical coiled-coil structure for collagen during 1954–55. Also, V. R. Sarma¹, a Madras University crystallographer was involved in the first ever crystal structure determination of the enzyme, lysozyme in UK by David Phillips and co-workers in 1965 and, also the first protein crystal structure, also an enzyme, ribonuclease A, determined in the US by Gopinath Kartha, J. Bello and D. Harker² in 1967. It is gratifying to note that the first protein ever to be crystallized has its structure unravelled eventually by Madras University crystallographers. They deserve to be congratulated upon this achievement.

JBU was also the first enzyme that provided the specific biological role for nickel. JBU along with other ureases forms a class of nickel-dependent metallo-enzymes synthesized by plants, some bacteria and fungi. They provide ammonia required for growth by catalysing the hydrolysis of urea into ammonia and carbon dioxide. Although the amino acid sequences of plant and bacterial ureases are closely related, some biological activities differ significantly. Plant ureases, but not bacterial ureases, possess insecticidal properties independent of their ureolytic activity. These contrasting biological properties and the fact that there was no structural information of plant ureases motivated Ponnuraj³ and his group at the Centre of Advanced Study in Crystallography and Biophysics, University of Madras to take up the
challenge to determine the crystal structure of plant ureases. They have recently reported the crystal structure of JBU (the first plant urease structure) with novel structural features critical for understanding its functions.

Plant ureases are made up of a single polypeptide chain in contrast to bacterial ureases, which consist of two or three polypeptides designated as α, β, and γ (Figure 1). In the crystals obtained by Karthe and his group, the crystallographic asymmetric unit contains one JBU molecule that packs against five neighbouring symmetry-related molecules, generating the biologically active hexamer, a dimer of trimers (Figure 2). This hexameric assembly with extensive intermolecular interactions, perhaps provides a structural rationale for the remarkable stability of JBU and its capacity to retain significant biological activity even after treatment with denaturing agents. In fact, this property has been exploited extensively for enzyme immobilization for industrial applications. Quaternary structural details now revealed by the crystal structure of JBU would prove to be valuable in the design of better carriers for enzyme immobilization.

Although crystal structures of microbial ureases from Klebsiella aerogenes, Bacillus pasteurii and Helicobacter pylori, as well as their inhibitor complexes were known, only preliminary results from X-ray analysis of JBU were available as far as plant ureases are concerned. Multiple isoforms and insolubility of JBU seemingly were the impediments for preventing the X-ray structure determination for so long. Karthe’s group has now successfully overcome these shortcomings.

The reported structure of JBU is quite similar to its bacterial counterparts, including the active-site architecture suggesting a conserved catalytic mechanism. As mentioned earlier, one of the biological functions that distinguishes plant and bacterial ureases concerns the former’s ability to act as an insecticide. The plant ureases, soybean and Jack bean, have been shown to possess insecticidal activity in insects with cathepsin B and cathepsin D-based digestive system. Interestingly, it was previously identified in canatoxin (isoform of JBU), that an internal 10 kDa peptide was released by hydrolysis of canatoxin by the enzyme cathepsin in the digestive system of susceptible insects, and this was found to be responsible for the entomotoxic effect.

Lack of this entomotoxic peptide in microbial ureases is attributed to their ineffectiveness as insecticides. By virtue of the makeup or composition of bacterial ureases (consist of two or three different chains; α, β, γ) as opposed to a single polypeptide chain in plant ureases, the linker peptide is missing in the former. Karthe and his group argue that this is probably responsible for lack of insecticidal property in bacterial ureases. In fact, mapping the 10 kDa insecticidal region of canatoxin onto the bacterial ureases, has shown that a region consisting of the C-terminus of the β-chain, and the N-terminus of the α-chain (Figure 1), corresponding to the region responsible for insecticidal activity, were not linked in bacterial ureases.

In the crystal structure of JBU, the 10 kDa insecticidal region (Gly 230–Val 320) consists of an α-helix, a long loop, another short helix and a χ-hairpin motif (Figure 3). It is known that the general mechanism of pore-forming toxins (PFTs) is to insert either an amphipathic α-helix or χ-hairpin to produce well-defined pores in the plasma membrane of the attacked cells. A subgroup of PFTs known as βPFTs was predicted to form β-barrels which insert into membranes to create pores. The individual χ-hairpin from one monomer pairs up with the neighbouring χ-hairpins of the other monomer to generate a β-barrel structure. Interestingly, the characteristic feature of these χ-hairpins is that they are largely amphipathic, with a large portion of hydrophobic residues and positively charged residues. In plant ureases, the putative membrane-disruptive χ-hairpin motif located in the 10 kDa entomotoxic peptide region, is highly conserved and exhibits an amphipathic character. Also, it has been shown that the recombinant form of the 10 kDa entomotoxic peptide region of JBU possesses membrane-disruptive ability on acidic lipid bilayers and it also forms aggregates. These observations and the structural features revealed in JBU, prompted Karthe’s group to propose that the amphipathic χ-hairpin located in the C-terminal region of the 10 kDa entomotoxic peptide of
plant ureases might form a membrane insertion β-barrel as in β-PFTs. This provides a natural explanation for bacterial urease (B. pasteurii) not being lethal to insects. They argue that although bacterial ureases contain this critical β-hairpin motif, they lack most of the N-terminal part of the β-hairpin motif. This is because in bacterial ureases the 10 kDa region is not intact as it is made up of two chains (α and β). As a result, the β-hairpin motif is contributed by the α-chain, whereas its N-terminal part comes from the β-chain (Figure 3). The authors further suggest that it is likely that the N-terminal part of the β-hairpin motif might be required for the overall stability of the putative transmembrane β-barrel and since this region is not attached to the β-hairpin motif, the overall stability of the β-barrel may be compromised in bacterial ureases.

JBU is of immense interest to the agricultural research community. Insect pests pose a major threat to majority of the commercially important crops, and transgenic crops with intrinsic pest resistance offer a promising alternative for chemical pesticides. Since plant ureases exhibit insecticidal property, understanding their three-dimensional structure has been vital. The structure of JBU reported by Karthe’s group has provided a structural basis for its endomotoxic activity, which might be relevant for development of insect-resistant transgenic plants.


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