Crystal structure of creatinine amidohydrolase (creatininase) from *Pseudomonas putida*

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This year we published the crystal structure of creatinine amidohydrolase (creatininase) from *Pseudomonas putida* [1] (PDB code 1Q3K) based on a Pt-MAD diffraction measurement with beamline BW7A at the EMBL outstation Hamburg in Sep. 2000. Creatininase is used in biosensors to determine the creatinine concentration in blood and thereby to detect renal dysfunctions [2]. The enzyme catalyses the following reaction:

\[
\text{Creatinine} + \text{H}_2\text{O} \xrightleftharpoons{\text{creatininase}} \text{Creatine}
\]

Two features of the creatininase structure were surprising against the background of the former literature about this enzyme [2]. In contrast to the established knowledge we found a hexameric rather than an octameric quaternary structure (Fig. 1) and we detected an active site with a double-zinc centre rather than a mono-zinc centre (Fig. 2).

Figure 1: Hexameric quaternary structure of creatininase with D₃ point symmetry. In contrast to this result of the crystal structure determination the older creatininase literature [2] states the enzyme to form an octameric quaternary structure.
Figure 2: Stereo picture of the dinuclear zinc center at the active site of creatininase. The density drawn in the picture is an OMIT-density calculated after a simulated annealing run in which the two zinc ions and the surrounding residues were left out. Hexameric quaternary structure of creatininase with D₃ point symmetry. The large piece of density is drawn with a sigma cutoff of 2.8, the small ones around the zinc ions with a sigma cutoff of 10. In contrast to our finding the result of previous studies addressing the metal content of creatininase had been that each creatininase monomer should contain only one zinc ion [2].

Based on our structure and the modelling of an analogue of the reaction transition state we proposed a reaction mechanism (Fig. 3) with a histidine as the central catalytic base rather than a carboxylate group like in dihydroorotase [3] and other cyclic amidohydrolases.

Figure 3: Reaction mechanism of creatininase proposed on the basis of the crystal structure [1]

In the future we will try to confirm these ideas by crystallization of enzyme/substrate complexes.

References