Current Topics in Biophysics, 2007, 30 (suppl. B) 36

NITRIC OXIDE DIFFUSION WITHIN NITRILE HYDRATASE REVEALS ARCHITECTURE OF ITS FUNCTIONAL CHANNEL

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Nitrile hydratase (NHase, EC. 4.2.1.84, CAS registration no. 82391–37–5) is a Co- or Fe-based metalloenzyme used in industrial biotechnology for large scale production of commonly used chemicals. NHases convert nitriles to amides. NHase selectivity and efficiency in amides production strongly depends on the central ion type. Interestingly, the Fe-type NHase is photosensitive. This photosensitivity is driven by nitric oxide, in dark conditions NO molecule is covalently bound to the active site and the catalytic reaction is impossible. The NO photodissotiation leads to the full recovery of catalytic abilities. The details of the catalytic mechanism, such as a role of a functional channel in structural selectivity remain unknown.

Nitric oxide diffusion path in the functional active site channel can reveal new information about its dynamics and key residues in the substrate recognition. Such knowledge can be useful in creation of more efficient enzymes and also enzymes catalyzing utilization of toxic nitriles.

Using molecular dynamics simulations (MD) results coupled with Locally Enhanced Sampling (LES) approach we were able to find crucial residues for NHase activity in the active site channel. Some residues form bulky barriers (βArg56 and βTyr76) for substrate/product diffusion and can be responsible for a substrate recognition. We found that water molecules are always present in the channel and a fast water exchange is possible. It seems that structural changes after NO dissociation/recombination are limited to the closest surrounding of the active site and its channel. Further investigations of NHase mutants dynamics are needed.

This research was supported by Polish Ministry of Education and Science, grant no. 2P04A 07229 and by UMK grant no. 356-F.