Poster Presentations

[MS5-P51] Recombinant fungal nitrilases - effect of reduction on their structure and function Ondřej Vaněk[1], David Illéš[1], Dorota Zawadová[1], Jan Bláha[1], Alicja B. Veselá[1,2], Ludmila Martínková[2]

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Nitrilases are versatile enzymes in biocatalysis, mediating the hydrolysis of diverse nitriles under mild conditions. Some of their products, notably hydroxy acids, such as mandelic, 3-hydroxyvaleric, or glycolic acid, are of significant industrial importance[1]. Arylaliphatic nitrilases (arylacetonitrilases; EC 3.5.5.1, EC 3.5.5.5) can transform, amongst other substrates, α-hydroxynitriles and α-aminonitriles, some of which are industrially important as the precursors of carboxylic acids and amides. For example, arylacetonitrilase from Labrenzia aggregata was used for the transformation of (R,S)-o-chloromandelonitrile into (R)-o-chloromandelic acid, a building block for the synthesis of Clopidogrel®, a platelet aggregation inhibitor [2]. As a result, numerous studies have focused on the screening and protein engineering of these enzymes. Most of the nitrilases studied and explored as biocatalysts have been bacterial in origin except for a few fungal and plant enzymes. According to both activity screens and gene database searches, filamentous fungi seem to be a rich source of nitrilases. We recently reported on the first arylacetonitrilases from fungi, that is from strains of Aspergillus niger and Neurospora crassa [1] and Arthroderma benhamiae [3]. Codon optimized synthetic genes for these enzymes were expressed in E. coli, purified and characterized as the first nitrilases from these genera.In this work, we studied these recombinant nitrilases from the point of view of their macromolecular assembly or oligomerization state. Wild-type nitrilase isolated from Aspergillus niger (and also other nitrilases from bacterial strains) forms spontaneously large helical structures, easily observable by electron microscopy. While the tendency to form these helical particles is decreased in recombinant enzyme, it still tends to form large and very heterogeneous oligomers of approx. 12-18 subunits as analyzed by gel filtration and analytical ultracentrifugation [4]. However, the oligomeric state dramatically changes upon protein reduction with dithiothreitol: while the nitrilase retains its enzymatic function, the previously covalently disulfidicly cross-linked molecules now form well defined monodisperse non- covalent complex of approx. 8 subunits. This behavior is the same for all bacterially expressed recombinant nitrilases from the three genera, pointing for a general tendency of these enzymes to form a large multi-subunit complexes. Therefore, new purification protocol in reductive environment was established which in the case of nitrilase from Arthroderma benhamiae already enabled successful crystallization of such a reduced enzyme preparation.

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