Side Chain Oxidation Modulates

Phenylalanine Hydroxylase Activity

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The monooxygenase phenylalanine hydroxylase (PheH, EC number 1.14.16.1) catalyzes the oxidation of L-phenylalanine to L-tyrosine at a non-heme iron active site. Molecular oxygen and the reductive co-factor tetrahydrobiopterin participate in the catalyzed redox reaction. Dysfunction of PheH prevents the commited step of phenylalanine degradation, leading to an accumulation of phenylalanine and in consequence to mental retardation, a well-studied genetic disease named phenylketonuria.

Recent studies highlight immune activation and inflammation to increase the ratio of phenylalanine to tyrosine in blood suggesting a downregulation of PheH [1]. As immune activation of macrophages is paralleled by the release of toxic reactive oxygen species, oxidative stress is discussed as chemical background for PheH dysfunction [2]. Furthermore, *in vitro* experiments showed disulfide reagents to modulate PheH activity [3].

Inspection of available X-ray structures revealed one apparent oxidizable site at two cysteine residues (Cys203, Cys334) distant from the active site. Mutations of both cysteine residues were shown to cause mild forms of phenylketonuria though their distance to the catalytic center [4,5]. Comparative molecular dynamics simulations were performed to analyze conformational differences of native PheH and the hypothetical protein oxidized at cysteine residues 203 and 334.

Starting from an X-ray structure of human PheH (PDB code 1J8U [6]) 100 ns of molecular dynamics simulations were carried out for each of the systems using the AMBER forcefield ff99SB [7]. Analyses of trajectories reveal, that cysteine oxidation modulates local dynamics in a loop region near the active site, which is experimentally known to show high flexibility and alterations of conformational behavior upon phenylalanine binding [8]. Increased flexibility of this region in the oxidized state is paralleled by a motion of the loop towards the active site reducing accessibility of the catalytic center providing a potential structural background for the oxidation-related inactivation of PheH.

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