PEP-INDUCED CONFORMATIONAL CHANGES IN EPSP-SYNTHASE FROM *MYCOBACTERIUM TUBERCULOSIS* DETERMINED BY HYDROGEN-DEUTERIUM EXCHANGE AND MASS SPECTROMETRY

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In plants and microorganisms the key aromatic compounds involved in primary metabolism are produced by the shikimate-pathway. Animals, in contrast, obtain their aromatic compounds from their diet. Thus, there is a keen interest in the shikimate-pathway enzymes as potential targets for the development of non-toxic herbicides and antimicrobial compounds. The EPSP-Synthase, the sixth enzyme in this pathway, transfers the enolpyruvyl group from PEP to shikimate-3-phosphate in order to form 5-enolpyruvyl-shikimate-3-phosphate and phosphate. The 3-D structure of this enzyme was determined and used to map PEP-induced conformational changes of the inactive MtEPSPS through the use of hydrogen amide exchange (HX), pepsin digestion and mass spectrometry (MS). The results showed that the binding of PEP resulted in an increasing of the structural organization of the enzyme, characterized a decreasing of solvent accessibility, especially in the region between the residues Val102-Phe170. A comparison in the level of deuterium incorporation of the pepsic peptides from the MtEPSPS in presence and absence of the PEP, revealed a more extensive deuteration of the free enzyme. Some regions showed significant differences in deuterium incorporation, especially those containing the amino acids residues responsible by the binding of the PEP (fragments 122-127/163-170/341-349/382-389/406-412). These results also demonstrate the capacity of HX/MS for examining the conformational changes of proteins dynamics in solution.

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