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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N backbone resonance assignments of the C-terminal domain of 5-enolpyruvylshikimate-3-phosphate synthase

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Biological context

5-Enolpyruvylshikimate-3-phosphate (EPSP) synthase catalyzes the formation of EPSP from shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP) in the biosynthesis of aromatic amino acids in bacteria, algae, fungi and plants, as well as apicomplexan parasites. Because the shikimate pathway is absent in mammals, EPSP synthase has been a target for herbicide, antibiotic and antiparasitic development. EPSP synthase is the primary site of action of glyphosate (GLP; *N*-phosphonomethyl glycine), a broad-spectrum post-emergence herbicide and the active ingredient of RoundUp^{TM}.

EPSP synthase, comprising 427 amino acids, is monomeric ($\sim$ 46 kDa) and folded into two distinctive globular N- and C-terminal domains of very similar size and symmetry connected by two linkers. In the absence of substrates, the two domains of EPSP synthase exist in an 'open' conformation, while they are thought to undergo changes to a 'closed' conformation upon substrate binding (Anderson et al., 1988). Recently, the X-ray crystal structures of liganded EPSP synthase have been determined to 1.5 Å resolution (Schönbrunn et al., 2001). The X-ray crystal structure of the ternary EPSPSS3Pglyphosate complex gives detailed interactions of S3P and GLP with substrate and/or inhibitor binding residues. However, the X-ray crystal structures have not sufficiently described the motion of domain closure of the enzyme. In addition, still little is known about the precise structural data of the PEP binding site, the conformational changes induced by PEP binding, and the difference between the PEP and glyphosate binding sites, which may provide crucial information about enzyme catalysis and mechanism. Therefore studies on the domain-specific contribution of EPSP synthase to substrate binding and catalysis may give an overall explanation of the enzyme in action. In order to address these issues, we have executed a 'divide and conquer' strategy: we have divided EPSP synthase into N- and C-terminal domains and are using the backbone assignments of the two domains in the assignment of the full-length protein. Previously, the chemical shift assignment, characterization, and structure calculation of the isolated N-terminal domain in the absence and presence of the substrate S3P have been carried out by our group (Stauffer et al., 2001a,b; Young et al., 2002). Here we report the backbone ^{1}H, ^{13}C, and ^{15}N chemical shift assignments for the C-terminal domain (211 residues, Mr 22 980 Da), the second half of the protein.

Methods and experiments

The isolated C-terminal domain (1–21, GG, 240–427, $\sim$ 23 kDa) was engineered by looping out the nucleotide sequence corresponding to the entire N-terminal domain (residues 22–239) from the plasmid containing full-length *aroA* gene. The resulting DNA fragment was cloned into the *Xba*I/*Bam*HI sites of the pET24b plasmid vector (Novagen). The isolated C-terminal domain was expressed in *E.coli* strain BL21(DE3). For the preparation of uniformly ^{15}N and ^{2}H, ^{13}C, ^{15}N labeled proteins, proteins were overexpressed and purified as described (Stauffer et al., 2001a,b). Sample purity ($\sim$95 %) and molecular weight were verified by SDS-PAGE and MALDI mass

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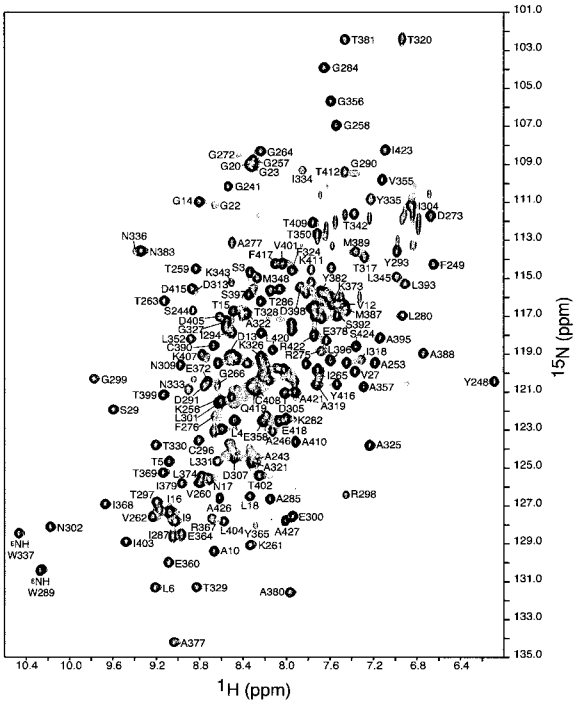

Figure 1. ^{15}N-HSQC of the C-terminal domain of EPSP synthase. Selected resonances are labeled according to the backbone assignments.

spectrometry. NMR samples were desalted and concentrated to $\sim$ 1 mM in 10 mM TrisHCl, pH 7.8, 3 mM NaN_3, 1 mM DTT, and 10% D_2O.

NMR data were recorded at 22 °C on a Varian Inova 500 NMR spectrometer. Backbone and C^β resonance assignments were made using sensitivity-enhanced HNCA, HN(CO)CA, HNCO, HN(CA)CO, HNCACB, 3D ^{1}H-^{15}N HMQC-(NOESY)-HMQC, ^{15}N-NOESY-HSQC experiments. NMR data processing was carried out using the NMRPipe (Delaglio et al., 1995), analyzed using the PIPP (Garrett et al., 1991) software packages. Proton chemical shifts (in ppm) were referenced to external TSP at 0 ppm, and ^{15}N and ^{13}C shifts were calculated relative to this according to Wishart et al. (1995).

Secondary structure was predicted from $^1H^\alpha$, C^α, CO chemical shifts using the chemical shift index (CSI) (Wüthrich et al., 1984).

Extent of assignments and data deposition

Figure 1 shows the assigned ^{15}N-HSQC spectrum of uniformly ^{15}N labeled C-terminal domain. Backbone assignments for the isolated C-terminal domain are virtually complete except for ^{15}N of 8 proline residues plus Met-1. A total of 96% of $^1H^N$ and ^{15}N resonances have been assigned. In total, 98% of $^{13}C^\alpha$, 95 % of $^1H^\alpha$, 95% of ^{13}CO resonances were assigned. Backbone amide resonances S245, S247, H310, I311, and W337 were not observed, presumably due to conformational exchange or rapid exchange with solvent. 3D ^{1}H-^{15}N HMQC-(NOESY)-HMQC spectra were recorded on a uniformly ^{15}N labeled sample and used to assign the degenerate backbone resonances. The secondary structure predictions based on CSI and short- and medium-range NOE data suggest that the first 20 residues fold into the rest of the C-terminal domain (residues 240–427), forming a native-like structure consisting of three βαβαββ folding units, as in the full-length protein (Schönbrunn et al., 2001). The chemical shifts ($^1H^N$, ^{15}N, $^{13}C^\alpha$, $^{13}C^\beta$, ^{13}CO and $^1H^\alpha$) have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5382.

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