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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of *Aquifex aeolicus* shikimate kinase in complex with the substrate shikimate

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Biological context

Shikimate pathway is a biosynthetic pathway with many branches that leads to the synthesis of aromatic amino acids, p-aminobenzoic acid and other essential nutrients (Bentley, 1990). The pathway is essential for microorganisms and plants and has never been found in vertebrates. Enzymes in the shikimate pathway are therefore excellent targets for developing antimicrobial agents (Davies et al., 1994) and herbicides (Kishore and Shah, 1988).

Shikimate kinase (SK) catalyzes the fifth reaction of the shikimate pathway, the transfer of γ-phosphate of ATP to shikimate to generate shikimate-3-phosphate. The structure of shikimate kinase from *Erwinia chrysanthemi* has been determined recently by crystallography (Krell et al., 1998), resulting in the elucidation of the interactions between the SK and MgADP, a product of the reaction, at atomic resolution. However, the electron density for shikimate was not clear enough for elucidating the interactions between the SK and shikimate.

We have overproduced the SK from the hyperthermophilic bacterium *Aquifex aeolicus* (AASK) in *E. coli*. Because of its high thermal stability, the enzyme is an excellent model for studying the structure and catalytic mechanism of SK by NMR. Here we report the sequential resonance assignment of the SK in complex with the substrate shikimate by multinuclear multidimensional NMR spectroscopy.

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Methods and results

The gene encoding AASK was cloned into the expression vector pET-17b by PCR from the genomic DNA of *Aquifex aeolicus* kindly provided by Dr. R. Huber (Universität Regensburg). The correct coding sequence of the cloned gene was verified by DNA sequencing. Uniformly ^{15}N- and ^{15}N/^{13}C-labeled proteins were produced by growing the *E. coli* strain BL21(DE3) containing the overexpression construct pET17b-AASK in a M9 medium with appropriate nitrogen and carbon sources. Production of AASK was induced by addition of IPTG to a final concentration of 0.5 mM when OD_{600} of the culture reached 0.8. The cells were harvested by centrifugation 4 h after induction and suspended in buffer A (20 mM Tris-HCl, pH 8.0). The bacterial suspension was then sonicated. The resulting lysate was centrifuged for 30 min at ∼27 000 g. The supernatant was loaded onto an Affi-Gel Blue column equilibrated with buffer A. The column was washed with buffer A and eluted with a 0.5–1 M linear NaCl gradient in buffer A. Fractions containing AASK were pooled, concentrated and loaded to a Sephadex G-75 column equilibrated with buffer A containing 300 mM NaCl. The column was developed with the same buffer. Pure SK fractions were pooled and concentrated to 10–20 ml. The concentrated SK was dialyzed against 1 mM HEPES (pH 7.5) and lyophilized.

NMR samples were prepared by dissolving lyophilized AASK in 100 mM sodium phosphate, pH 7.0 (pH meter reading without correction for isotope effects), in $H_2O/^2H_2O$ (9/1). The samples contained ∼2 mM AASK (oxidized, with a disulfide bond formed by C10 and C110) and ∼22 mM shikimate. All NMR data were acquired at 30 °C on a Varian Inova 600 spectrometer with a triple-resonance probe-

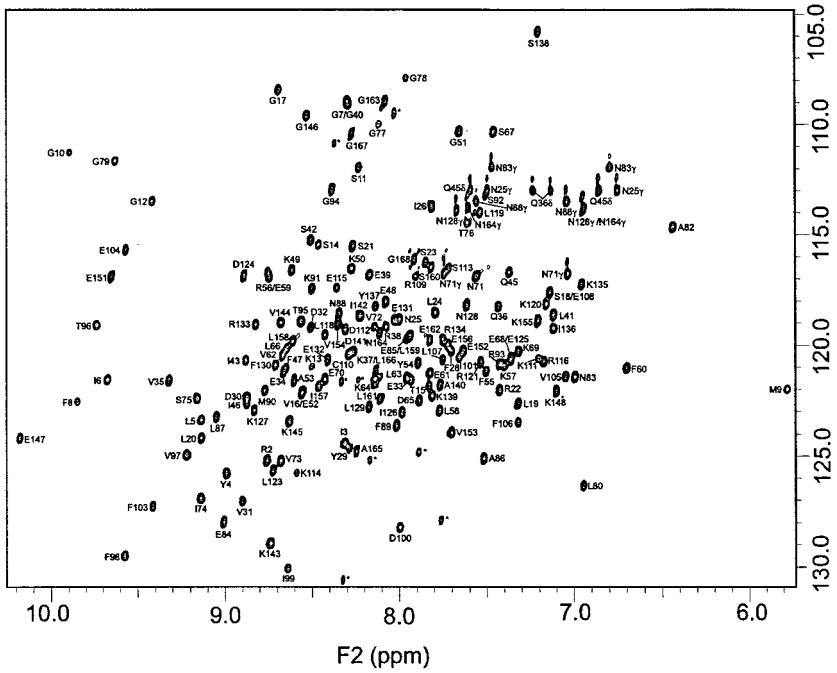

Figure 1. ^{1}H-^{15}N HSQC spectrum of *Aquifex aeolicus* SK in complex with shikimate recorded at 30 °C and a ^{1}H frequency of 600 MHz with coherence selection by pulsed field gradients and sensitivity enhancement. The NMR sample contained ∼2 mM SK and ∼22 mM shikimate in 100 mM sodium phosphate buffer, pH 7.0. Sequential assignments are indicated with the one-letter amino acid code and residue number. G81 (100.9, 7.34 ppm) is not shown. The peaks with asterisks may be due to impurities.

head. The NMR experiments performed have been described in recent excellent reviews (Bax and Grzesiek, 1993; Kay, 1995), including sensitivity-enhanced 2D ^{1}H-^{15}N HSQC, 3D ^{15}N-edited TOCSY-HSQC, NOESY-HSQC (150 ms mixing time), HNCACB, CBCA(CO)NH, HNCO and HCCH-TOCSY. All the NMR data were processed by NMRPipe (Delaglio et al., 1995) and analyzed with FELIX 95 (Biosym). The backbone resonance assignment was achieved mainly by the combined analysis of the HNCACB and CBCA(CO)NH data. The side-chain resonances were identified mainly by the analysis of HCCH-TOCSY. Aromatic ring resonances were assigned based on the analysis of 2D homonuclear TOCSY and NOESY and heteronuclear ^{1}H-^{13}C HSQC data.

Extent of assignments and data deposition

All backbone amide resonances were observed in the ^{1}H-^{15}N HSQC spectrum and have been assigned (Figure 1). Of the other backbone resonances, 100% have been assigned for C^{α}, 98% for H^{α}, and 94% for C'. Overall, ∼95% aliphatic side-chain resonances have been assigned. The assignments have been deposited in the BioMagResBank database under accession number 4722.

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