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Letter to the Editor: Backbone resonance assignment of an aminoglycoside-3′-phosphotransferase type IIa

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Biological context

Antibiotic resistance against the aminoglycoside/aminocyclitol family is mostly achieved through enzymatic covalent modification of these compounds. These modifications block the antibiotic's interaction with its target, the bacterial ribosomal aminoacyl-tRNA site (Llano-Sotelo et al., 2002). Three classes of modifying enzymes have been identified in all clinically relevant bacteria of both Gram-positive and Gram-negative origin (Wright, 1999): (1) aminoglycoside-*O*-phosphotransferases (APHs), (2) aminoglycoside-*O*-adenyltransferases (ANTs) and (3) aminoglycoside-*N*-acetyltransferases (AACs).

APHs catalyze the ATP-dependent phosphorylation reaction, where the γ-phosphate of ATP is transferred to specific aminoglycoside hydroxyl groups. Several families of APHs have been classified with respect to their reaction and substrate specificity.

We have initiated the structural characterization of kanamycin-3′-*O*-phosphotransferase type IIa (APH(3′)IIa, kanamycin kinase) originating from the *Klebsiella pneumoniae* transposon *Tn5*. This enzyme is a 29 kDa (264 aa) monomeric single-chain protein. Here we report the sequence-specific NMR backbone assignment of kanamycin kinase.

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Methods and experiments

Kanamycin kinase was purified from a kanamycin kinase accumulating *Pseudomonas oleovorans* strain harbouring plasmid pBHR81 (derivative of pBBR1MCS-2; Rehm et al., 1998; Kovach et al., 1995). *P. oleovorans* was grown in M9 media at 30 °C. [^{15}N]-ammonium chloride, [^{13}C]-sodium acetate, [^{13}C]-glucose, [^{2}H, ^{13}C]-glucose, [3,3-$^{2}H_2$, ^{13}C]-2-ketobutyrate, [3-^{2}H, ^{13}C]-2-ketoisovalerate and 99.9% or 95% $^{2}H_2O$ were used as isotope sources for production of protein samples with different isotope labelling schemes. Purification of kanamycin kinase to over 95% purity as judged from gel electrophoresis was achieved by ion exchange chromatography on Q-Sepharose and subsequent size exclusion chromatography on Superdex-75 columns. The NMR buffer contained 20 mM BIS-TRIS (pH 6,5), 20 mM sodium chloride, 60 mM potassium chloride, 10 mM dithiothreitol, 100 μM EDTA, 5% $^{2}H_2O$ and 0.02% sodium azide. The following kanamycin kinase samples of approx. 1 mM protein concentration (5 mm NMR tubes, 320 μl, Doty susceptibility plugs) were used for NMR spectroscopy: [*U*-^{2}H, *U*-^{15}N]-, [*U*-^{2}H, *U*-^{13}C, *U*-^{15}N]-, [60–70%-^{2}H, *U*-^{13}C, *U*-^{15}N]- and [*U*-^{2}H, *U*-^{13}C, *U*-^{15}N]/[Val-$\gamma^{1/2}$-, Leu-$\delta^{1/2}$, Ile-δ^{1}-^{1}H]-kanamycin kinase.

NMR experiments were carried out on Varian Unity *INOVA* instruments operating at 750 or 900 MHz proton frequency. All spectra were recorded at 25 °C. The following spectra were used for backbone resonance assignment: 2D ^{1}H-^{15}N-TROSY-HSQC, 2D ^{1}H-^{13}C-ct-HSQC, 3D TROSY-

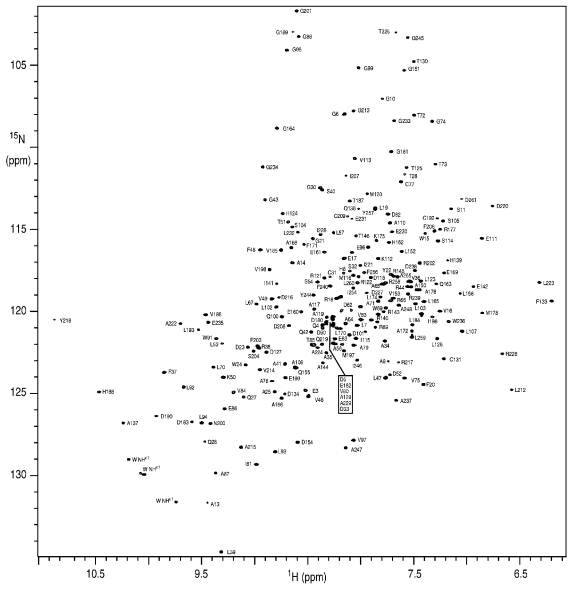

Figure 1. 2D ^{1}H-^{15}N TROSY-HSQC of [*U*-^{2}H, *U*-^{13}C, *U*-^{15}N]-kanamycin-3′-*O*-phosphotransferase type IIa (APH(3′)II, kanamycin kinase) recorded at 900 MHz. Sequence specific assignments of the cross peaks are indicated. Amino acids are designated using single letter code.

type HNCACB, 3D TROSY-type HNCA, 3D TROSY-type HNCO, 3D HNHA and 3D ^{1}H-^{15}N-TROSY-NOESY with diagonal peak suppression (Meissner and Sørensen, 2000; 175 ms mixing time). Spectra were processed using Vnmr (Varian, Inc.) or NMRPipe (Delaglio et al., 1995) and analyzed using XEASY (Bartels et al., 1995).

Extent of assignments and data deposition

Interpretation of the TROSY-type triple resonance backbone assignment experiments and the 3D HNHA spectrum lead to the sequence-specific assignment of 93% of the backbone amides (Figure 1) and 95, 94, 88 and 73% of the C^{α}, C^{β}, C′ and H^{α} resonances, respectively. In particular, the segments comprising N58-L60, M148/E149, L241-L243 and D250-R253 could not be assigned. Evaluation of the obtained C^{α}, C^{β}, C′ and H^{α} chemical shifts by the CSI program (Wishart and Sykes, 1994) lead to the prediction of ten α-helical and five β-sheet segments longer than three residues. Inspection of the 3D ^{1}H-^{15}N TROSY-NOESY spectrum with diagonal suppression (Meissner and Sørensen, 2000) for key long-range and sequential amid/amid NOE correlations confirms the occurrence of eight α-helices and a five-stranded antiparallel β-sheet within the N-terminal 100 residues. The topology of the β-strands is I-II-III-V-IV. The pattern of secondary structure found here is in excellent agreement with a crystallographic structure of a homologous APH(3′)IIIa enzyme (Hon et al., 1997) and of a crystallographic structure of APH(3′)IIa published very recently (Nurizzo et al., 2003). The availability of both the backbone resonance assignments and the three-dimensional structure of kanamycin kinase will be instrumental to now analyse by NMR the interaction of the enzyme with a wide variety of ligands including new inhibitors.

The backbone ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited at BioMagResBank (www.bmrb.wisc.edu) under accession number BMRB-5721.

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