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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N assignment of a bleomycin resistance protein in its native form and in a complex with Zn^{2+} ligated bleomycin

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Biological context

Bleomycin (Blm) is a glycopeptide from actinomycetes (Umezawa et al., 1966) which binds a single transition metal ion, e.g., Fe^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} (Sugiura, 1980). In a reductive environment and in the presence of oxygen, the Blm(Fe^{2+}) complex cleaves DNA (Sausville et al., 1976). The capability of Blm to cleave DNA is used in clinical drug combination to treat human cancers (Blum et al., 1973) despite some severe secondary effects on the lungs (pulmonary fibrosis). Some organisms exhibit a Blm resistance. In prokaryotes, there are two mechanisms of Blm resistance: one is the *N*-acetylation of Blm by transferase which is present for example in *Streptomyces verticillus* (Sugiyama et al., 1994). The other one is the Blm sequestration by a Blm resistance protein (BRP) as found in *Streptoalloteichus hindustanus (Sh)* (Gatignol et al., 1988; Drocourt et al., 1990). It has been shown that the secondary effects of Blm on the lungs during cancer treatment can be reduced by a *Sh* BRP gene therapy (Tran et al., 1997).

The crystal structure of *Sh* BRP (124 residues) exhibits a dimer organisation which is in agreement with biophysical solution studies (Dumas et al., 1994). In their study the authors propose a structural model of the Blm(Cu^{2+})•BRP complex based on the individual crystal structures, and electrostatic and steric considerations. However, so far no direct experimental data on a Blm•BRP complex has been obtained. We have therefore initiated NMR studies of a diamagnetic Blm(Zn^{2+})•BRP complex in order to determine its solution structure. In this note, we report complete backbone assignments (H^N, N, C^α, and C') for native *Sh* BRP, as well as ^{1}H, ^{13}C, and ^{15}N backbone and side chain assignments for the Blm(Zn^{2+})•BRP complex.

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Methods and results

Uniformly ^{15}N and ^{13}C/^{15}N-labelled BRP was obtained by growing *E. coli* HMS(DE3) in minimal media containing 1 g/l $^{15}NH_4Cl$ and 2 g/l [$^{13}C_6$]-glucose, for the ^{13}C-labelled sample only. BRP was then purified from the periplasmic fraction. NMR samples were prepared at a concentration of 1 mM in 20 mM MES buffer (90% H_2O, 10% D_20) at pH = 6.5, 100 mM NaCl, and 0.01% NaN_3. First, Blm obtained as a gift from Rhône Poulenc was mixed with $ZnSO_4$ (Fluka) to form a Blm(Zn^{2+}) complex. Blm(Zn^{2+}) was then added to native BRP in a 1:1.1 BRP:Blm ratio to form the Blm(Zn^{2+})•BRP complex.

All NMR experiments were performed on Varian INOVA 600 and INOVA 800 spectrometers, both equipped with a triple-resonance (^{1}H, ^{15}N, ^{13}C) probe and shielded z-gradients. The sample temperature was set to 40 °C. Quadrature detection in the indirect dimensions of the multidimensional experiments was achieved by the echo/antiecho detection scheme for ^{15}N, and by the TPPI-States method for ^{1}H and ^{13}C. All triple-resonance experiments used the pulse sequences provided by the Varian protein pack (available at ftp site: ftp.nmr.varian.com). The spectral

widths and carrier frequencies (in parentheses) were set for ^{1}H to 12 ppm (4.63 ppm), for ^{15}N to 30 ppm (117.4 ppm), for $^{13}C^{aliph}$ to 65 ppm (35 ppm), for $^{13}C'$ to 20 ppm (175 ppm), and for $^{13}C^{\alpha}$ to 30 ppm (56 ppm). All chemical shifts were referenced with respect to DSS for ^{1}H and ^{13}C, and liquid NH_3 for ^{15}N following the IUPAC recommendations (Markley et al., 1998).

For sequential backbone assignment 3D ^{15}N-edited NOESY-HSQC and TOCSY-HSQC spectra were recorded on ^{15}N-labelled samples of native BRP and the Blm(Zn^{2+})•BRP complex at 800 MHz ^{1}H frequency. Data sets were acquired with 512(^{1}H) × 60(^{15}N) × 120(^{1}H) complex points and 8 scans per (t_1,t_2) increment. The NOESY and TOCSY mixing times were set to 150 ms and 50 ms, respectively. Additional triple-resonance experiments were performed at 600 MHz ^{1}H frequency on the $^{13}C/^{15}N$-labelled samples for unambiguous sequential backbone assignment, and for the assignment of the backbone ^{13}C resonances. For native BRP, 3D HNCA and HNCO spectra were recorded, and for the Blm(Zn^{2+})•BRP complex, a set comprising 3D HNCA, HNCO, CBCANH, and CBCA(CO)NH experiments was used. The typical time domain data size for these experiments was 512(^{1}H) × 45(^{15}N) × 60(^{13}C) complex points acquired with 8 scans per (t_1,t_2) increment. ^{1}H and ^{13}C side chain assignments (except for aromatic nuclei) of the Blm(Zn^{2+})•BRP complex were accomplished using a set of three 3D triple-resonance experiments: H(C)CH-TOCSY acquired with 512(^{1}H) × 128(^{13}C) × 128(^{1}H) complex points, H(C)C(CO)NH-TOCSY, and (H)C(CO)NH-TOCSY, acquired with 512(^{1}H) × 42(^{15}N) × 80(^{1}H or ^{13}C) complex points.

Data processing and peak picking were performed using FELIX program version 97.0 (MSI Technologies). After signal apodisation using squared cosine functions and zero-filling the time-domain data were Fourier transformed to final 3D matrices of typically 512 × 128 × 256 data points.

Extent of assignments and data deposition

The assigned ^{1}H -^{15}N HSQC spectrum of Blm(Zn^{2+}) complexed BRP is shown in Figure 1. Unambiguous assignment of backbone resonances (H^N, N, C', and C^{α}) was obtained for all residues, except for M1, L4, and Q56 in native BRP and for M1 and L4 in the Blm(Zn^{2+})•BRP complex. In addition, side chain assignments for 99% of the aliphatic ^{13}C and 96% of the aliphatic ^{1}H resonances were obtained for the Blm(Zn^{2+})•BRP complex.

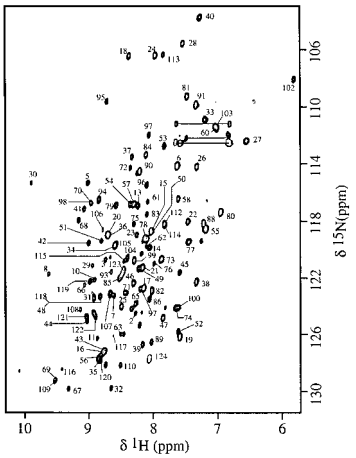

Figure 1. ^{1}H-^{15}N HSQC spectrum of the Blm(Zn^{2+})•BRP complex.

The ^{1}H, ^{13}C, and ^{15}N assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession numbers BMRB-4785 (BRP) and BMRB-4786 (Blm(Zn^{2+})• BRP).

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