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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N backbone resonance assignments for TEM-1, a 28.9 kDa β-lactamase from *E. coli*

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Biological context

The production of β-lactamases by bacteria is one of the most efficient and prevalent mechanisms of resistance to β-lactam antibiotics (Therrien and Levesque, 2000). Among the class A β-lactamases, TEM-1 (263 residues, Mw 28907 Da) is the most commonly found and is responsible for the resistance to β-lactam antibiotics of various pathogenic bacteria. This enzyme catalyzes very efficiently the hydrolysis of the amide bound in the β-lactam ring of antibiotics that include the widely used penicillins and cephalosporins. Its catalytic action is characterized by a simple acylenzyme pathway in which the acylenzyme intermediate is hydrolyzed by a water molecule in order to regenerate the active site for the next turnover (Strynadka et al., 1992). To date, more than 100 naturally occurring mutants of TEM-1 have been recorded (http://www.lahey.org/Studies/temtable.asp). Despite the large amount of data obtained by various techniques such as X-ray crystallography (Minasov et al., 2002; Strynadka et al., 1992) or molecular dynamics simulations (Oliva et al., 2003; Diaz et al., 2003), the acylation mechanism of TEM-1 and other class A β-lactamases remains unclear and conjectural. As resistant strains are still appearing concomitantly with the development of new antibiotics and β-lactamase inhibitors, more information on the mechanism of action of these proteins are needed.

In order to better understand finer details behind this mechanism, we have undertaken the NMR characterization of TEM-1. Here we report the first backbone assignment for a class A β-lactamase (TEM-1). This assignment is the first step of several NMR structural and dynamical studies of class A β-lactamases and β-lactamases complexes. This work will give a better understanding of the mechanism of action of these enzymes and could facilitate the development of new therapeutic weapons against resistant bacteria.

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Methods and experiments

Labeling, expression and purification of TEM-1

The plasmid pET-24 containing the *bla* gene coding for the mature protein fused to the signal peptide ompA was used to transform *Escherichia coli* BL21(DE3) for protein expression (Sosa-Peinado et al., 2000). The gene sequence was verified by automatic sequencing and it is the same as the wild type except for the third N-terminal residue which is a Gly instead of a Glu. ^{15}N, $^{15}N/^{13}C$ and $^{2}H/^{15}N/^{13}C$ uniformly labeled protein samples were prepared by growing cells on M9 minimal medium containing $^{15}NH_4Cl$, ^{13}C or $^{2}H/^{13}C$ glucose and/or 100% D_2O (Cambridge Isotope Labs, Andover, MA). Expression and purification was as described in Sosa-Peinado et al. (2002). The yields using this protocol were 40–80 mg/l of highly pure TEM-1 in its mature form (residues 26–288).

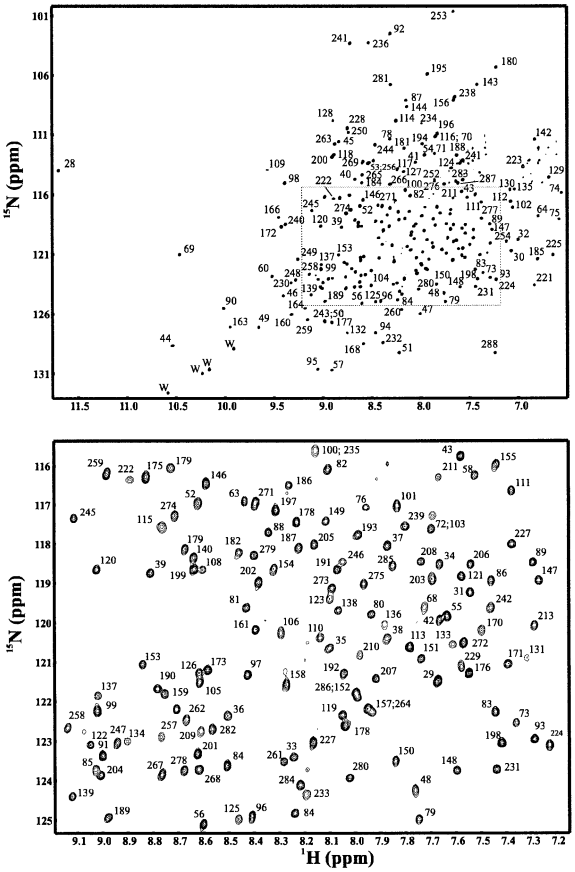

Figure 1. 600 MHz 2D ^{1}H, ^{15}N-TROSY-HSQC spectrum of TEM-1 β-lactamase. Numbers indicate residue numbers. W indicates Trp side-chain. The sequence numbering is sequential, from H^{26} to W^{288}.

NMR spectroscopy

For collection of NMR spectra, the protein was lyophylised after extensive dialysis against water and was dissolved to ∼0.7 mM in a buffer containing 25 mM $NaPO_4$ pH 6.6, 3.0 mM imidazole, a cocktail of protease inhibitors, 0.1% sodium azide, 0.1 mM DSS for internal referencing and 90% H_2O/10% D_2O. NMR experiments were performed at 30 °C on Varian INOVA 600 MHz and 800 MHz spectrometers. All spectra were processed with nmrPipe (Delaglio et al., 1995) and analyzed in NMRView (Johnson and Blevins, 1994). Resonance assignments were derived manually from TROSY versions (Pervushin et al., 1997) of a series of 3D experiments from Varian's BioPack (Varian Inc, Palo Alto): HNCO, HNCA, HNCACB, CBCA(CO)NH, HN(CO)CA, and HN(CA)CO.

Extent of assignments and data deposition

The 2D ^{1}H, ^{15}N-HSQC spectrum of triply-labeled TEM-1 (Figure 1) exhibits a very good dispersion of the proton and nitrogen resonances. In total, 99.6% of all backbone ^{1}HN and ^{15}N (for the non-proline residues), 99.2% of all ^{13}Cα, 98.8% of all ^{13}Cβ and 99.9% of all ^{13}C′ assignments were obtained. The missing backbone assignments are: ^{13}C′-Ser^{106}, ^{13}C′-Ile^{173}, ^{13}Cβ-Ser^{203}, ^{13}Cα/^{13}Cβ-Lys^{215}, and ^{1}HN/^{15}N-Ala^{237}. It was not possible to obtain ^{13}C assignments for the first residue (His^{26}) since it is followed by a Proline. Some missing assignments and several broad resonances correspond to residues that are proximal in the 3D structure. Secondary structure prediction based on chemical shift index (Wishart and Sykes, 1994) is in good agreement with the crystallographic data. Backbone chemical shifts have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under accession number 6024.

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References

Delaglio, F., Grzesiek, S., Vuister, G.W., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Díaz, N., Sordo, T.L., Merz, Jr. K.M. and Suárez, D. (2003) *J. Am. Chem. Soc.*, **125**, 672–684.

Johnson, B.A. and Blevins, R.A. (1994) *J. Biomol. NMR*, **4**, 603–614.

Minasov, G., Wang, X. and Shoichet, B.K. (2002) *J. Am. Chem. Soc.*, **124**, 5333–5340.

Oliva, M., Dideberg, O. and Field, M.J. (2003) *Proteins*, **53**, 88–100.

Pervushin, K., Riek, R., Wider, G. and Wüthrich, K. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 12366–12371.

Sosa-Peinado, A., Mustafi, D. and Makinen, M.W. (2000) *Protein Exp. Purif.*, **19**, 235–245.

Strynadka, N.C.J., Adachi, H., Jensen, S.E., Johns, K., Sielecki, A., Betzel, C., Sutoh, K. and James, M.N.G. (1992) *Nature*, **359**, 700–705.

Therrien, C. and Levesque, R.C. (2000) *FEMS Microbiol. Rev.*, **24**, 251–262.

Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.