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Sequence-specific ^{1}H, ^{13}C and ^{15}N assignment of the TMP-resistant dihydrofolate reductase mutant DHFR(F98Y) in the ternary complex with TMP and NADPH

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Biological context

The treatment of antibacterial infections is hampered by increasing resistance against virtually all antibiotics in clinical use. One of these antibiotics is trimethoprim (TMP), which acts on dihydrofolate reductase (DHFR) (Roth et al., 1962). Dale et al. (1997) showed that a single amino acid substitution in wild-type DHFR, resulting in DHFR(F98Y), is the molecular origin of the TMP resistance of *Staphylococcus aureus*. The virtually complete sequence-specific assignment of the 158-residue protein DHFR(F98Y) provides a basis for investigations on the structural background of the observed TMP resistance, including comparisons with the X-ray crystal structure of the wild-type protein.

Methods and Results

For the protein preparation, *Escherichia coli* M15-(pREP), transformed with the DHFR(F98Y) expression plasmid, was grown at 37 °C. Purification included affinity chromatography with BlueSepharose (Pharmacia), elution with a salt gradient, and subsequent gel filtration. Thus, 77% of the DHFR(F98Y) in the cell extract was recovered. The yields of pure DHFR(F98Y) were 6 mg per liter of culture in minimal medium, and 20 mg in rich medium.

In a search for the solution conditions to be used for the structure determination, the pI of the protein was determined to be 5.7 and light scattering revealed that the protein is monomeric in aqueous solution at NMR concentrations. CD measurements showed that the ternary complex with TMP and NADPH is the most stable form, and that the protein is not stable below pH 5.8. Therefore, DHFR(F98Y) was studied in the complex with TMP and NADPH at pH 7.3 and 298 K. The protein concentration was 2.0 mM, 1.0 mM and 1.3 mM in phosphate-buffered saline (68.5 mM NaCl, 1.35 mM KCl, 0.75 mM KH_2PO_4, 4.0 mM Na_2HPO_4, pH 7.3, 90% H_2O/10% D_2O) for the unlabeled, ^{15}N-labeled and $^{13}C/^{15}N$-labeled samples, respectively. A doubly labeled 1.3 mM DHFR-(F98Y) sample in 100% D_2O was obtained by five repeats of exchanging deuterated phosphate-buffered saline by ultrafiltration through a 5 kDa MWCO membrane (Millipore, Biomax). A sample volume of 220 µl was used in Shigemi tubes.

For NMR spectroscopy we used a Bruker DMX 600 MHz spectrometer, with quadrature detection in the phase-sensitive mode by TPPI (Marion and Wüthrich, 1983) or States–TPPI (Marion et al., 1989). The time-domain data were zero-filled to the next power of 2 and multiplied by phase-shifted sine bells (De Marco and Wüthrich, 1976). Linear prediction was applied for constant-time evolution periods.

The assignment of the polypeptide backbone was based on 3D HNCO, 3D CBCA(CO)NH and 3D CBCANH experiments (Bax and Grzesiek, 1993). Out of 149 expected signals, 148 were observed by HNCO, and these $^{15}N/^{1}H^{N}$ chemical shift pairs were transferred to the 3D CBCA(CO)NH and 3D CBCANH spectra. Analysis of strips along the ^{13}C dimension taken at these positions was used for the assignment of the other backbone heavy nuclei and C^{β}, using the program XEASY (Bartels et al., 1995). H^{α} and H^{β} assignments were deduced from 3D HBHA(CO)NH, and complete assignments of the CH_n moieties in nonaromatic side chains were obtained with 3D HCCH-TOCSY (Fig. 1), 3D H-DIPSY(CO)NH and 3D C-DIPSI(CO)NH (Bax and Grzesiek, 1993). Proline

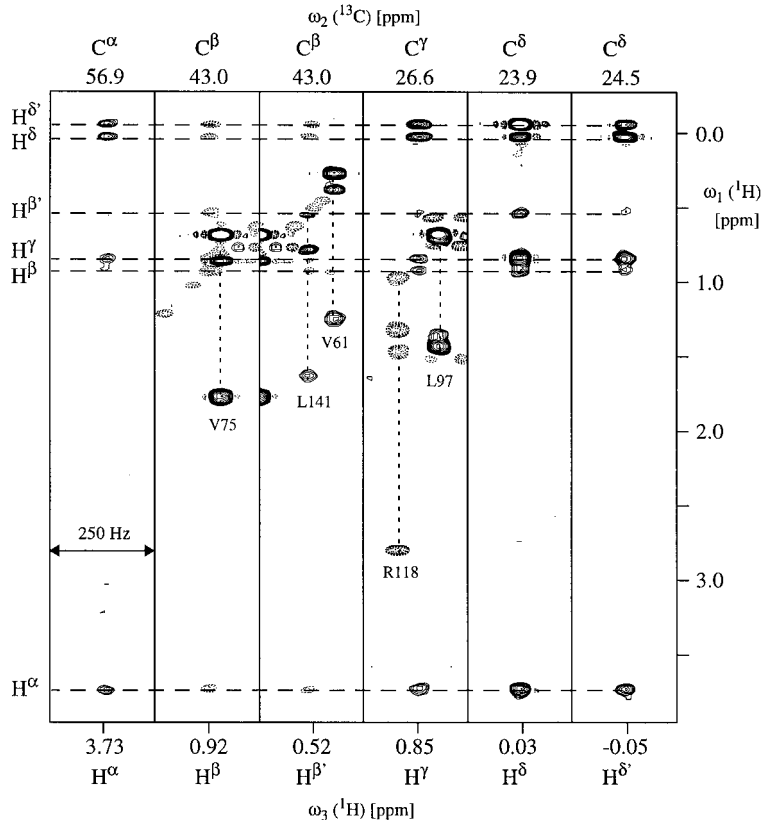

Fig. 1. Strips along $\omega_1(^1H)$ from 3D HCCH-TOCSY (1.3 mM $^{13}C/^{15}N$-labeled DHFR(F98Y) complexed with TMP and NADPH in phosphate-buffered saline (see text), 298 K, $\tau_m = 22.6$ ms, $64 \times 72 \times 768$ complex points, $t_{1max}(^1H) = 16.4$ ms, $t_{2max}(^{13}C) = 12.0$ ms and $t_{3max}(^1H) = 52.1$ ms), taken at the $^1H/^{13}C$ chemical shifts of Leu[34]. Solid and dotted contours represent positive and negative peaks, respectively. Dashed horizontal lines are placed at the $\omega_1(^1H)$ chemical shift positions of Leu[34]. Signals from other residues are connected by dotted vertical lines and identified by the amino acid one-letter symbol and the sequence number.

spin system identification was based on NOEs, starting from the H^δ and C^δ chemical shifts identified in a 2D ct-[^{13}C,^1H]-HSQC spectrum. Aromatic proton spin systems were assigned using 3D ^1H-TOCSY-relayed-ct-[^{13}C,^1H]-HMQC (Zerbe et al., 1996), and sequence-specific assignments were based on NOEs with $C^\beta H_2$ (Wüthrich, 1986). NH_n groups of Asn, Gln, Arg, Trp and His were observed in [^{15}N,^1H]-HSQC, and sequence-specific assignment was achieved using intraresidual NOEs in 3D ^{15}N-resolved NOESY (Wüthrich, 1986).

Extent of assignments and data deposition

All ^1H, ^{15}N and ^{13}C polypeptide backbone resonances were assigned, except Thr[1] NH_2, Asn[56] H^N and Arg[57] C'. Among the nonaromatic side-chain protons only δCH_2 of Lys[45] and γCH_2 of Met[101] remained unassigned, but for part of the methylene and isopropyl groups only a single line was observed and the question of degeneracy was not further investigated. The assignment of the aromatic protons is complete, except that those of the eight imidazole rings of His were not assigned, and the assignments for 5 of the 10 Phe rings were incomplete. Among the labile side-chain protons, those of all four Gln and all six Asn, $H^{\varepsilon 1}$ of the two Trp, two imidazole NH out of the six His, and five out of the six H^ε of Arg were assigned.

The ^1H, ^{13}C and ^{15}N chemical shifts for DHFR(F98Y) at pH = 7.0 and T = 298 K have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4027.

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