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Sequence-specific ^{1}H, ^{15}N and ^{13}C assignments of the periplasmic chaperone FimC from *Escherichia coli*

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Biological context

The periplasmic chaperone FimC is a monomeric, basic 23 kDa protein of 205 amino acid residues (Klemm, 1992) that mediates the assembly of type-I pili in *Escherichia coli*. Type-I pili are oligomeric protein complexes anchored on the surface of the bacterium and are required for the attachment of *E. coli* cells to host cell surfaces that are rich in mannose (Klemm, 1992). The determination of the three-dimensional structure of FimC should provide novel insights into the mechanism of the chaperone-assisted assembly of bacterial pili on the molecular level.

Methods and results

Thermal denaturation of FimC monitored by circular dichroism (CD) spectroscopy revealed a melting point of 63.5 °C at pH= 5.0. Hence, the NMR experiments were performed with the unlabeled, ^{15}N-labeled or $^{13}C_1{}^{15}$N-labeled protein at pH= 5.0 and T=38 °Con Bruker AMX600, Bruker DRX500 and Varian U750+ spectrometers equipped with four channels. Sequence-specific NMR assignments were obtained based in part on sequential NOE connectivities (Wüthrich, 1986) established with 3D ^{15}N-resolved [^{1}H-^{1}H]-NOESY and 3D ^{15}N-resolved [^{1}H-^{1}H]-TOCSY, and in part on heteronuclear scalar coupling connectivities established using 3D *ct*-HNCA (Grzesiek and Bax, 1993), 3D HNCACB (Wittekind and Mueller, 1993), and 3D $H^{\alpha/\beta}$ $C^{\alpha/\beta}$ (CO)NHN (Szyperski et al., 1996) spectra (Figure 1). Stretches of sequentially connected spin systems were mapped onto the amino acid sequence of FimC with the program MAPPER (P. Güntert, unpublished), which is based on statistical analysis of $^{13}C^{\alpha/\beta}$ chemical shifts in a data base of experimental shifts in globular proteins. The chemical shifts of the αCH-βCH$_n$ fragments provided the starting point for nearly complete ^{1}H and ^{13}C assignments of all CH$_n$ moieties in non-aromatic side chains, using a 3D HCCH-COSY spectrum (Grzesiek and Bax, 1993). ^{1}H spin systems of the aromatic rings of Trp, Tyr and Phe were identified in a 2D ^{1}H-TOCSYrelayed *ct*-[^{13}C, ^{1}H]-HMQC spectrum (Zerbe et al., 1996) with the doubly labelled sample, and in 2D [^{1}H, ^{1}H]-NOESY and 2D [^{1}H, ^{1}H]-TOCSY spectra recorded with a sample of unlabelled FimC in D_2O. Sequence-specific assignments were then obtained via NOEs between the aromatic ring and the β- and/or α-protons (Wüthrich, 1986). Backbone carbonyl resonances were assigned with a 3D *ct*-HNCO experiment (Grzesiek and Bax, 1993). The side chain amide proton resonances of Asn and Gln were assigned by intraresidual NOEs (Wüthrich, 1986). The guanidino $^{1}H^{\epsilon}$ and $^{1}H^{\eta}$ resonances of Arg were obtained based on scalar coupling connectivities delineated with a 2D HE(NE)HGHH spectrum (Pellecchia et al., 1997b). The assignments of the individual side chain carboxyl resonances of Asp and Glu were obtained using a 2D H(C)CO_2 experiment (Pellechia et al., 1997a). Asn and Gln side chain carbonyl resonances were assigned in a 2D *ct*-H(N)CO spectrum (Grzesiek and Bax, 1993). Stereospecific assignments for the isopropyl methyls of Val and Leu were obtained using biosynthetically directed fractional ^{13}C labelling (Senn et al., 1989) and 2D [^{13}C,^{1}H]-COSY.

Extent of assignments and data deposition

All ^{1}H, ^{15}N and ^{13}C polypeptide backbone resonances were assigned except for those of Gly 1, for which

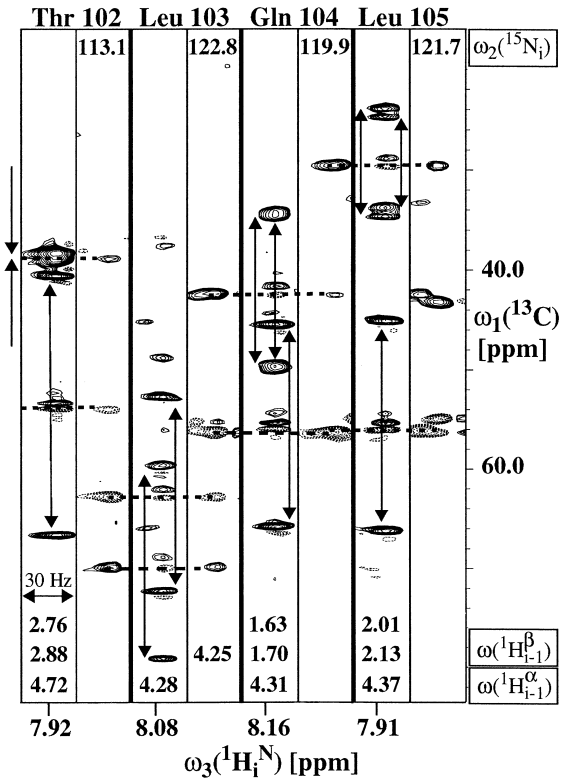

Figure 1. Contour plot of pairs of $[\omega_1(^{13}C^{\alpha/\beta}), \omega_3(^1H^N)]$ strips from a 3D HNCACB spectrum (right) and a 3D $H^{\alpha/\beta}C^{\alpha/\beta}$(CO)NHN spectrum (left). The spectra were obtained with a 1 mM solution of $^{15}N/^{13}C$-labelled FimC (90% H_2O/10% D_2O T= 38 °C, pH = 5.0) on a Bruker AMX600 spectrometer. For the 3D HNCACB spectrum 59 (t_1) × 30(t_2) × 512(t_3) complex points were acquired with $t_{1max}(^{13}C^{\alpha/\beta})$ = 6.6 ms, $t_{2max}(^{15}N)$= 18.3 ms, $t_{3max}(^1H^N)$= 62.5 ms. For the 3D $H^{\alpha/\beta}C^{\alpha/\beta}$(CO)NHN spectrum 82($t_1$) × 32($t_2$) × 512($t_3$) complex points were acquired, $t_{1max}(^{13}C^{\alpha/\beta})$= 6.6 ms, $t_{2max}(^{15}N)$= 19.5 ms, $t_{3max}(^1H^N)$ = 62.5 ms. In the 3D $H^{\alpha/\beta}C^{\alpha/\beta}$(CO)NHN spectrum, the offset during the proton chemical shift evolution was set to 2.8 ppm and the scaling factor (Szyperski et al., 1996) was set to 1.7 so that $t_{1max}(^1H^{\alpha/\beta})$ = 11.2 ms. If we indicate with Δ the in-phase splitting along $\omega_1(^{13}C^{\alpha/\beta})$ in ppm, and with ν_C and ν_H the Larmor frequencies of 150.90 MHz for ^{13}C and 600.13 MHz for ^1H, the $^1H^{\alpha/\beta}$ chemical shifts indicated in ppm at the bottom of the strips are calculated according to $\omega(^1H^{\alpha/\beta})$= 2.8 ppm + $[(\Delta \times \nu_C)/(\nu_H \times 2 \times 1.7)]$ ppm for protons with a chemical shift > 2.8 ppm, and $\omega(^1H^{\alpha/\beta})$ = 2.8 ppm − $[(\Delta \times \nu_C)/(\nu_H \times 2 \times 1.7)]$ ppm for protons with a chemical shift <2.8 ppm (Szyperski et al., 1996). The strips were taken at the $^1H^N/^{15}N$ positions of Thr 102 to Leu 105, as indicated at the bottom of the figure and at the top of the 3D HNCACB strips. Dotted and solid contours indicate negative and positive peaks, respectively. Sequential connectivities are indicated by dashed horizontal lines. In the strips from the 3D $H^{\alpha/\beta}C^{\alpha/\beta}$(CO)NHN spectrum the $^1H^\alpha$ and $^1H^\beta$ chemical shifts of the preceding residue as extracted from the in-phase splitting (vertical arrows) are also reported (residue 101 is Asn).

only C^α and H^α were assigned. In the amino acid side chains the following resonances of ^{13}C and of non-labile hydrogens remained unassigned: $C^{\gamma 1}$ of Leu 98, 103, 105 and 120; $CH_2^{\delta/\epsilon}$ of Lys 16, 58, 61, 44, 60, 88, 95, 97, 130 and 199; CH_3^ϵ of Met 57, 93 and 170; CH_2^γ and C^δ of Glu 27 and 80; C^γ of Asn 86; $CH_2^{\gamma/\delta}$ of Pro 168 and 198; $C^{\gamma/\delta}$ of Gln 126. The assignment of the aromatic rings is complete. The labile side chain proton resonances of Asn, Gln and Trp, and H^ϵ of Arg were completely assigned. Out of the 11 Arg, the assignments for the guanidino H^η resonances were obtained for residues 8, 66, 79, 110, 132, 134 and 160. For 1 α-CH_2, 37 β-CH_2 and 12 γ-CH_2 groups only one proton resonance was observed. For 2 Val and 4 Leu only one proton resonance and one carbon resonance were observed for the isopropyl methyl groups. For 59 methylene protons, stereospecific assignments were obtained using the routines HABAS and GLOMSA (Güntert et al., 1997). For the isopropyl methyls, stereospecific assignments were obtained for 9 out of the 13 Val and for 14 out of the 23 Leu. Individual proton assignments were obtained for 21 of the 22 NH_2 groups of Asn and Gln. The ^1H, ^{13}C and ^{15}N chemical shifts for FimC have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4070.

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