Eur Biophys J (1996) 24: 179–184

European Biophysics Journal

Resonance assignment and structural analysis of acid denatured *E. coli* [U-^{15}N]-glutaredoxin 3

Use of 3D ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC

Kerstin Nordstrand, Hannes Ponstingl, Arne Holmgren, Gottfried Otting

Department of Medical Biochemistry and Biophysics, Karolinska Institute, S-171 77 Stockholm, Sweden

Received: 5 September 1995 / Accepted: 1 November 1995

Abstract. Virtually complete sequence specific ^{1}H and ^{15}N resonance assignments are presented for acid denatured reduced *E. coli* glutaredoxin 3. The sequential resonance assignments of the backbone rely on the combined use of 3D F_1-decoupled ROESY-^{15}N-HSQC and 3D ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC using a single uniformly ^{15}N labelled protein sample. The sidechain resonances were assigned from a 3D TOCSY-^{15}N-HSQC and a homonouclear TOCSY spectrum. The presented assignment strategy works in the absence of chemical exchange peaks with signals from the native conformation and without ^{13}C/^{15}N double labelling. Chemical shifts, ^{3}J(αH, NH) coupling constants and NOEs indicate extensive conformational averaging of both backbone and side chains in agreement with a random coil conformation. The only secondary structure element persisting at pH 3.5 appears to be a short helical segment comprising residues 37 to 40.

Key words: Protein denaturation – Folding – NMR – Resonance assignments – ^{15}N labelling

Introduction

The resonance assignment of denatured proteins is complicated by the exceptionally small chemical shift dispersion in the ^{1}H NMR spectrum (Wüthrich 1994). It has been shown that complete resonance assignments can be obtained using ^{15}N/^{13}C doubly labelled protein samples (Alexandrescu et al. 1994; Logan et al. 1994) or the chemical exchange peaks in equilibria between native and denatured protein conformations combined with uniform ^{15}N enrichment (Neri et al. 1992 a, b; Zhang et al. 1994). In the absence of chemical exchange peaks or ^{13}C/^{15}N double labelling, the resonance assignments are far more difficult. In a recent study of partially denatured human ubiquitin using a uniformly ^{15}N labelled sample, some of the backbone ^{1}H resonances were left unassigned (Stockman et al. 1993). The present work demonstrates that the use of the recently published 3D ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC experiment (Zhang et al. 1994) enables the resonance assignment of larger, fully denatured proteins under conditions where chemical exchange peaks cannot be observed and ^{13}C labelling is not available. The assignment strategy is illustrated with the virtually complete ^{1}H resonance assignment of acid denatured, uniformely ^{15}N enriched *E. coli* glutaredoxin 3 ([U-^{15}N]-glutaredoxin 3), a protein with 82 amino acid residues (Åslund et al. 1994, 1996). The sequence specific resonance assignments present the basis for an evaluation of the randomness of the denatured conformation.

Abbreviations: HSQC, heteronuclear single quantum coherence; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, two-dimensional NOE spectroscopy; ROE, nuclear Overhauser effect in the rotating frame; ROESY, two-dimensional ROE spectroscopy; TOCSY, total correlation spectroscopy; TPPI, time proportional phase incrementation

Correspondence to: G. Otting (Fax: +46-8-33 52 96, Tel.: +46-8-728 6804, e-mail: go@mfn.ki.se)

Experimental

[U-^{15}N]-glutaredoxin 3 was prepared by expression on minimum medium containing ^{15}N ammonium sulfate as the only nitrogen source. The protein was used in its reduced form with no disulfide bridges present. The NMR measurements were performed with a 7 mM solution of *E. coli* glutaredoxin 3 in 90% H_2O/10% D_2O, pH 3.5 at 28 °C. All data were recorded on a Bruker DMX-600 NMR spectrometer operating at a ^{1}H frequency of 600 MHz.

A 3D F_1-decoupled ROESY-^{15}N-HSQC spectrum was recorded using the experimental scheme of Messerle et al. (1988) with the ROE mixing spin-lock replacing the NOE mixing sequence and the first spin-lock purge pulse. In addition, a selective inversion pulse on the α-protons together with a hard 180°(^{1}H) pulse was inserted in the middle of the evolution period to decouple the αH signals in the F_1 dimension (Brüschweiler et al. 1988; Otting et al. 1990). The experiment was implemented with an eight step

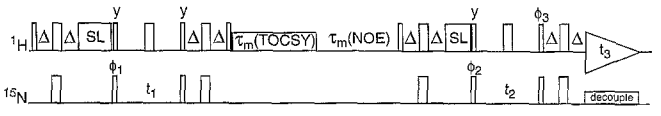

Fig. 1. Pulse sequence for the ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC experiment. Narrow and wide bars denote 90° and 180° pulses, respectively. Spin-lock pulses are denoted SL. Pulse phases are along the x-axis unless indicated differently. The delay Δ is equal to $1/(4^1J(^{15}N,^1H))$. Phasecycle: $\Phi_1 = 4(x,-x)$; $\Phi_2 = 2(x,x,-x,-x)$; $\Phi_3 = 4(x),4(-x)$; receiver = x,-x,-x,x,-x,x,x,-x. The phases of all ^{15}N pulses preceeding t_1 and t_2, respectively, are incremented according to the States-TPPI scheme (Marion et al. 1989). The water signal is suppressed by the spin-lock pulses combined with homospoil pulses during τ_m(NOE) which also support the coherence order selection

phase cycle, where the phases of the inversion pulses in the middle of t_1 were cycled between x and y, and one of the 90°(^{15}N) pulses was cycled between x and -x. The spectrum was recorded in 52 h using a ROE mixing time of 85 ms, $t_{1max} = 48$ ms, $t_{2max} = 46$ ms, $t_{3max} = 190$ ms, and a data size of 63, 64 and 1024 complex points in F_1, F_2 and F_3, respectively. The water signal was suppressed by a spin-lock purge pulse of 2 ms. The constant delays in the HSQC part were set to 2.8 ms.

The 3D ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC experiment was originally designed with gradients and selective flip-back pulses to avoid water saturation (Zhang et al. 1994). In the case of acid denatured glutaredoxin 3, saturation transfer from the water signal is unimportant, since the amide proton exchange rates are near their minimum at pH 3.5 (Wüthrich 1986). Furthermore, resolution is more critical than signal-to-noise. Therefore we used a simplified version of the experiment which can be recorded with fewer scans per FID (Fig. 1). The data were recorded in 63 h using the pulse sequence of Fig. 1 with τ_m(TOCSY) = 49 ms, τ_m(NOE) = 250 ms, $\Delta = 2.8$ ms, $t_{1max} = 46$ ms, $t_{2max} = 46$ ms, $t_{3max} = 190$ ms, and a data size of 64, 64 and 1024 complex points in F_1, F_2 and F_3, respectively. The clean CITY mixing scheme was used during τ_m(TOCSY) (Briand and Ernst 1991). The spin-lock purge pulses were 2 ms and 500 μs long (Fig. 1). A total of six homospoil pulses of 2 ms duration were applied during τ_m(NOE) at regular intervals.

$^3J(\alpha H, NH)$ coupling constants were measured from the in-phase multiplet splittings of the cross peaks in ^{15}N-HSQC spectra (Szyperski et al. 1992).

Results and discussion

E. coli glutaredoxin 3 is reversibly denatured in aqueous solution by lowering the pH to 4.0 and below. Although the native and denatured forms coexist at about pH 4.5, the exchange is too slow for the observation of chemical exchange cross-peaks. The sequence specific resonance assignments of the denatured state could not be obtained from two-dimensional ^{1}H NMR data because of spectral overlap. 3D ROESY-^{15}N-HSQC and 3D TOCSY-^{15}N-HSQC spectra alone proved to be insufficient for the sequential resonance assignments as well. Because of near or full degeneracy between the amide and α-protons of sequentially neighboring amino acid residues, sequential d_{NN} and $d_{\alpha N}$ connectivities (Wüthrich 1986) are frequently unresolved, while sequential $d_{\beta N}$ connectivities are absent or too weak to be observed.

Although the chemical shifts of all but one backbone amide proton are within a range of 0.7 ppm, the ^{1}H-^{15}N cross-peaks are, with very few exceptions, resolved in a ^{15}N-HSQC experiment. The 3D ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC experiment (Zhang et al. 1994) makes full use of the ^{15}N chemical shift dispersion by presenting the information on the sequential connectivities in a 3D NMR experiment, where the indirectly detected dimensions are the ^{15}N chemical shifts of the amide groups of sequentially neighboring amino acid residues. However, when the experiment was applied to an equilibrium mixture of native and denatured drkN SH3 domain (Zhang et al. 1994), only scarce correlations were obtained for the denatured form, which is largely due to the chemical exchange between the two conformational states. With acid denatured glutaredoxin 3 which is in slow chemical exchange with the native state, this experiment yielded the sequential connectivities between all non-proline residues, except for the few cases where the ^{15}N chemical shifts of the sequentially neighboring residues are degenerate. Together with the connectivities observed in the 3D ROESY-^{15}N-HSQC experiment, complete backbone ^{1}H and ^{15}N assignments were obtained.

The NOE between the α proton of residue i and the amide proton of the sequentially following amino acid residue is the only sequential connectivity which is always expected to be observable irrespective of the prevailing secondary structure (Wüthrich 1986). Similarly, the α and the amide proton of an amino acid residue are scalarly coupled, which is the basis for the observation of intraresidual αH-NH cross peaks in TOCSY. The sequence of TOCSY mixing followed by NOE mixing in the 3D ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC experiment directs the magnetization transfer from the amide proton of residue i to the α proton of the same residue via the scalar coupling and further to the amide proton of residue i+1 via the sequential αH-NH NOE.

Figure 2 shows a comparison between the sequential connectivities observed in the 3D F_1-decoupled ROESY-^{15}N-HSQC spectrum and the 3D ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC spectrum for the polypeptide segment between Ala 59 and Leu 68 of acid denatured glutaredoxin 3. As is apparent from the 3D F_1-decoupled ROESY-^{15}N-HSQC spectrum (Fig. 2 A), the α-proton resonances of Ala 59 and Gln 60, Gly 63 and Gly 64, and Asp 66 and Asp 67 overlap, making it difficult to trace the sequential connectivities. The corresponding sequential connectivity pathway in the 3D ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC spectrum amino acid residues are linked by two connectivities. The major connectivities are derived from the following magnetization transfer: ^{15}N(i) during t_1, transfer from NH(i) to αH(i) during τ_m(TOCSY), transfer from αH(i) to NH(i+1) during τ_m(NOE), ^{15}N(i+1) during t_2, and NH(i+1) during t_3. The magnetization transfer for the second set of connectivities follows the path-

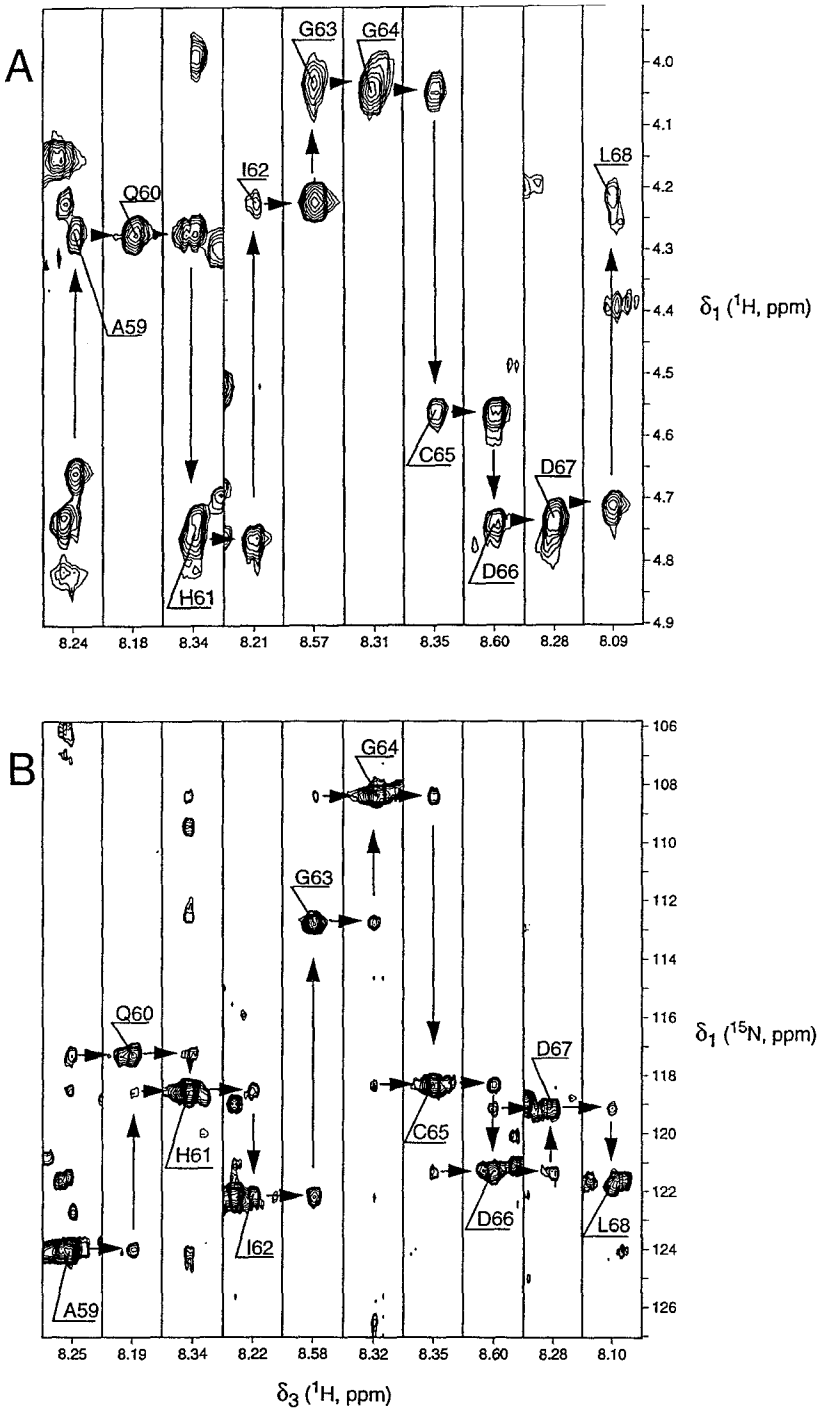

Fig. 2 A, B. Sequential assignment of residues 59 to 68 of a 7 mM solution of acid denatured *E. coli* glutaredoxin 3 in 90% H_2O/10% D_2O, pH 3.5, 28 °C. Arrows identify the sequential assignment pathways. **A** Selected strips taken from the 3D F_1-decoupled ROESY-^{15}N-HSQC spectrum. Intraresidual $\alpha H(\delta_1)$-$N(\delta_2)$-$NH(\delta_3)$ cross-peaks are identified with the one-letter symbol of the amino acid residue and the sequence number. **B** Selected strips taken from the ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC experiment. Intraresidual $N(\delta_1)$-$N(\delta_2)$-$NH(\delta_3)$ cross-peaks are identified as in **A**

way: ^{15}N(i) during t_1, NH(i) during τ_m(TOCSY), transfer from NH(i) to NH(i–1) during τ_m(NOE), ^{15}N(i–1) during t_2, and NH(i–1) during t_3. These connectivities are usually quite weak and often missing, because the NOEs for sequential d_{NN} connectivities are weaker than for $d_{\alpha N}$ connectivities.

Figure 3 shows an overview of the sequential backbone connectivities obtained for acid denatured glutaredoxin 3 from the 3D F_1-decoupled ROESY-^{15}N-HSQC and 3D ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC spectra. The sequential connectivities combined from both experiments cover the complete amino acid sequence, except at proline residues which have no amide proton and at the step Glu 41 – Glu 42. The $d_{\alpha\delta}$ connectivities with the proline residues missing in Fig. 3 were observed for all proline residues in a two-dimensional ROESY spectrum. Residues 41 and 42 have very similiar ^{15}N and ^{1}H chemical shifts leading to connectivities which overlap in all spectra.

The resonances of the side-chains were identified from a 3D TOCSY-^{15}N-HSQC and a 2D TOCSY spectrum. Because of relatively slow ^{1}H relaxation in denatured glutaredoxin 3, the signal-to-noise ratio in the TOCSY experiments was excellent and enabled the correlation of the backbone amide protons with the most remote protons of

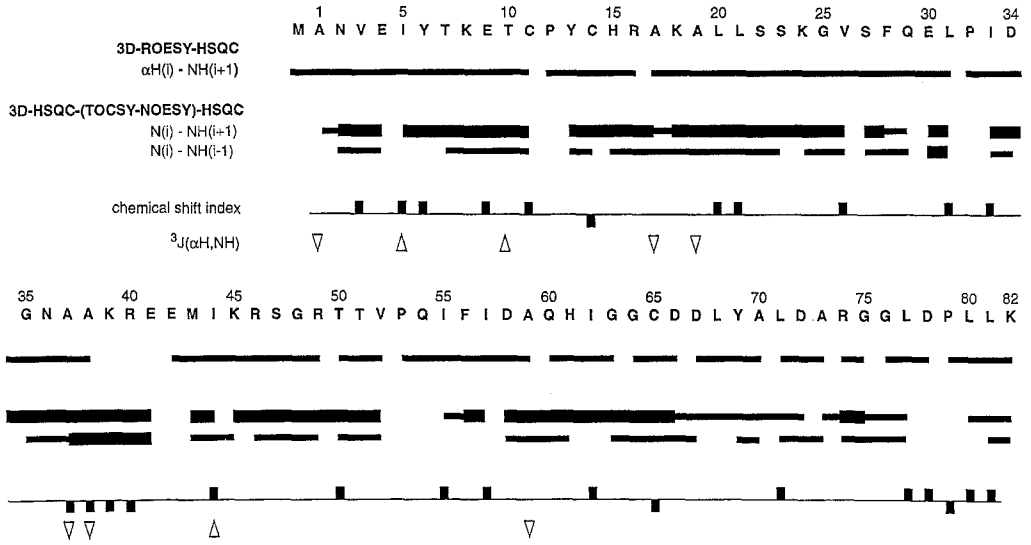

Fig. 3. Overview of the sequential connectivities observed in the 3D F_1-decoupled ROESY-^{15}N-HSQC and the 3D ^{15}N-HSQC-(TOCSY-NOESY)-^{15}N-HSQC spectra. The intensities of the sequential cross-peaks in the 3D HSQC-(TOCSY-NOESY)-HSQC spectrum are represented by strong and narrow lines. N(i)-NH(i+1) and N(i)-NH(i–1) indicate connectivities through αH(i)-NH(i+1) and NH(i)-NH(i–1) NOEs, respectively. The missing connectivities with the δ protons of the proline residues were observed in a 2D ROESY experiment. Underneath the connectivity lines, the chemical shift index of the α protons (Wishart et al. 1992) is indicated for each residue. Furthermore, residues for which ^{3}J(αH, NH) coupling constants larger than 8 Hz and smaller than 6 Hz were measured are identified by up-arrows and down-arrows, respectively. All other residues show ^{3}J(αH, NH) coupling constants between 6 and 8 Hz, except for glycine and proline residues and the residues 1, 36 and 80 for which this coupling constant could not be measured by the method used (see Experimental)

the amino acid sidechains (data not shown). The ^{1}H chemical shifts of all residues are very similar to the random coil chemical shifts observed with small peptides (Table 1). Consequently, the side-chain protons could be assigned confidently from the TOCSY data by reference to the random coil chemical shifts. Where only a single resonance was observed for methylene protons, chemical shift degeneracy is most likely the cause. The only remaining ambiguities are for sidechains with overlapping resonances, such as the side-chains of phenylalanine, some of the leucine β and γ protons, and some of the isoleucine methyl groups. For some protons, such as the methyl protons of the methionine residues, no TOCSY cross-peaks could be detected.

The only secondary structure element persisting in acid denatured glutaredoxin 3 seems to be a helical segment comprising residues 37 to 40, which is indicated by the αH chemical shifts using the chemical shift index of Wishart et al. (1992), strong NH(i)-NH(i+1) NOE connectivities in the 3D HSQC-(TOCSY-NOESY)-HSQC spectrum, and small ^{3}J(αH, NH) coupling constants (Fig. 3). Yet, not a single αH(i)-NH(i+3) NOE could be detected throughout the entire polypeptide chain either because of spectral overlap or too weak intensities. Remarkably, all ^{3}J(αH, NH) coupling constants smaller than 6 Hz are from alanine residues, and only some isoleucine residues and one threonine residue show a ^{3}J(αH, NH) coupling constant larger than 8 Hz (Fig. 3). These results correlate with the propensities for α-helices and β-sheets of the respective residues (Chou and Fasman 1974). NOE data observed with unfolded FK506 binding protein suggested previously that the preferred backbone conformation in denatured proteins correlates with the Chou-Fasman rules of secondary structure prediction (Logan et al. 1994). Our data on chemical shifts and coupling constants in acid denatured glutaredoxin 3 confirm this notion. Yet, all ^{3}J(αH, NH) coupling constants measured are confined to the range 5.3 to 8.1 Hz indicating extensive conformational averaging of the polypeptide chain with average conformations outside the range of stable α-helical or extended secondary structure. Most notably, only few sidechain protons deviate in their chemical shifts from random coil values (Table 1). The observation of sequence dependent chemical shifts for the proline side chains is most likely a manifestation of the simple fact that the side chain is linked to the backbone via two covalent bonds. For all other amino acid residues, deviations of the side chain chemical shifts from the random coil values are observed, when they are followed by an aromatic residue or when the residues carry aromatic side chains themselves. In these situations, ring currents would be expected to have a significant impact on the chemical shifts.

In summary, acid denatured *E. coli* glutaredoxin 3 is characterized by extensive conformational averaging of both the polypeptide backbone and the amino acid side chains. In particular, no sign of residual tertiary structure was found. Therefore, the acid denatured state is an adequate starting point for studies of the folding kinetics into the native conformation. Comparative studies of the conformation of *E. coli* glutaredoxin 3 under different denaturing conditions are in progress which make use of the assignment strategy established here.

Acknowledgement. We thank Fredrik Åslund for help with the preparation of [U-^{15}N]-glutaredoxin 3. This work was supported by the Swedish Natural Science Research Council (Project 10161), the Swedish Cancer Society (Project 961), the P. A. Hedlund Foundation, and the K. A. Wallenberg Foundation.

Table 1. Backbone chemical shifts of acid denatured *E. coli* glutaredoxin 3 in H_2O at pH 3.5 and 28 °C

Residue	Chemical shift (ppm)				Others
	^{15}N	NH	αH	βH	
Met 0			4.16	2.20	γCH_2 2.66
Ala 1	126.6	8.74	4.42	1.42	
Asn 2	118.7	8.56	4.72	2.78, 2.84	δNH_2 6.93, 7.62
Val 3	120.0	8.12	4.15	2.10	γCH_3 0.94
Glu 4	124.0	8.40	4.36	1.96	γCH_2 2.34, 2.39
Ile 5	121.6	8.07	4.14	1.78	γCH_2 1.13, 1.36; $\gamma CH_3/\delta CH_3$ 0.82
Tyr 6	124.0	8.27	4.71	2.95, 3.11	δH 7.15; εH 6.82
Thr 7	115.9	8.04	4.33	4.22	γCH_3 1.20
Lys 8	123.0	8.27	4.31	1.79, 1.87	γCH_2 1.45; δCH_2 1.71; εCH_2 3.03
Glu 9	120.8	8.36	4.44	2.01, 2.13	γCH_2 2.47
Thr 10	114.9	8.20	4.34	4.24	γCH_3 1.20
Cys 11	122.8	8.36	4.75	2.90	
Pro 12			4.42	1.96, 2.23	γCH_2 1.80, 1.87; δCH_2 3.68, 3.74
Tyr 13	119.2	8.00	4.57	2.98, 3.08	δH 7.11; εH 6.84
Cys 14	120.1	8.08	4.43	2.87	
His 15	121.1	8.54	4.72	3.18, 3.31	δH 7.31; εH 8.63
Arg 16	122.4	8.26	4.34	1.76, 1.84	γCH_2 1.65; δCH_2 3.19; εNH_2 7.18
Ala 17	125.6	8.40	4.30	1.41	
Lys 18	120.7	8.29	4.25	1.77, 1.83	γCH_2 1.45; δCH_2 1.72; εCH_2 3.03
Ala 19	124.9	8.27	4.31	1.38	
Leu 20	121.5	8.23	4.37	1.64	γH 1.60; δCH_3 0.89, 0.94
Leu 21	122.8	8.23	4.41	1.69	γH 1.63; δCH_3 0.89, 0.94
Ser 22	116.0	8.26	4.52	3.87, 3.94	
Ser 23	117.8	8.36	4.50	3.89, 3.94	
Lys 24	122.5	8.31	4.36	1.78, 1.89	γCH_2 1.43; δCH_2 1.68; εCH_2 3.01; ηNH_2 7.53
Gly 25	109.5	8.35	3.99		
Val 26	118.8	7.99	4.18	2.06	γCH_3 0.87, 0.90
Ser 27	119.0	8.35	4.49	3.81	
Phe 28	122.1	8.26	4.65	3.05, 3.14	γH 7.25
Gln 29	121.5	8.22	4.31	1.92, 2.03	γCH_2 2.29; εNH_2 6.87, 7.52
Glu 30	121.4	8.30	4.36	1.97, 2.10	γCH_2 2.45
Leu 31	124.7	8.27	4.64	1.63	γH 1.57; δCH_3 0.93
Pro 32			4.49	2.06, 2.27	γCH_2 1.93, 2.04; δCH_2 3.66, 3.85
Ile 33	120.3	8.20	4.15	1.87	γCH_2 1.21, 1.55; γCH_3 0.92; δCH_3 0.89
Asp 34	122.4	8.45	4.73	2.84, 2.91	
Gly 35	109.4	8.42	3.98		
Asn 36	118.6	8.34	4.70	2.85	δNH_2 6.99, 7.67
Ala 37	124.1	8.25	4.20	1.45	
Ala 38	121.5	8.16	4.24	1.43	
Lys 39	119.1	8.02	4.23	1.84, 1.88	γCH_2 1.45, 1.52; δCH_2 1.71; εCH_2 3.02; ηNH_2 7.56
Arg 40	120.6	8.13	4.25	1.71, 1.87	γCH_2 1.63; δCH_2 3.23; εNH 7.25
Glu 41	119.7	8.28	4.28	2.08	γCH_2 2.49
Glu 42	120.0	8.21	4.29	2.09	γCH_2 2.49
Met 43	120.1	8.18	4.43	2.11	γCH_2 2.58, 2.67
Ile 44	121.5	8.04	4.09	1.90	γCH_2 1.21, 1.54; γCH_3 0.91; δCH_3 0.87
Lys 45	124.4	8.29	4.32	1.82, 1.87	γCH_2 1.42, 1.52; δCH_2 1.71; εCH_2 3.03
Arg 46	121.8	8.32	4.40	1.83, 1.92	γCH_2 1.69; δCH_2 3.22; εNH 7.24
Ser 47	116.4	8.31	4.49	3.94	
Gly 48	110.4	8.41	4.03		
Arg 49	120.2	8.23	4.46	1.80, 1.90	γCH_2 1.66; δCH_2 3.23; εNH 7.21
Thr 50	115.4	8.30	4.48	4.27	γCH_3 1.24
Thr 51	116.8	8.23	4.41	4.22	γCH_3 1.20
Val 52	123.6	8.20	4.47	2.11	γCH_3 0.96, 1.01
Pro 53			4.42	2.06, 2.31	γCH_2 1.88, 2.00; δCH_2 3.71, 3.91
Gln 54	120.9	8.43	4.26	1.97	γCH_2 2.32; εNH_2 6.91, 7.53
Ile 55	120.8	8.04	4.16	1.77	γCH_2 1.12, 1.38; $\gamma CH_3/\delta CH_3$ 0.82
Phe 56	124.0	8.32	4.71	2.97, 3.11	γCH 7.23
Ile 57	122.7	8.08	4.10	1.82	γCH_2 1.15, 1.48; γCH_3 0.88; δCH_3 0.85
Asp 58	122.6	8.40	4.63	2.79, 2.92	
Ala 59	124.0	8.23	4.25	1.40	
Gln 60	117.3	8.17	4.25	1.96, 2.03	γCH_2 2.33; εNH_2 6.86, 7.51
His 61	118.6	8.35	4.74	3.16, 3.29	δH 7.29; εH 8.61
Ile 62	122.1	8.21	4.21	1.78	γCH_2 1.20, 1.49; γCH_3 0.92; δCH_3 0.86
Gly 63	112.8	8.57	4.00, 4.04		

Table 1. Continued

Residue	Chemical shift (ppm)				Others
	^{15}N	NH	αH	βH	
Gly 64	108.5	8.31	4.03		
Cys 65	118.3	8.34	4.53	2.95	
Asp 66	121.3	8.61	4.71	2.82, 2.90	
Asp 67	119.1	8.28	4.68	2.82, 2.87	
Leu 68	121.8	8.10	4.20	1.52	γH 1.39; δCH_3 0.82
Tyr 69	118.6	7.95	4.54	2.95, 3.11	δH 7.13; εH 6.84
Ala 70	123.5	7.95	4.28	1.39	
Leu 71	120.0	8.03	4.29	1.66	γH 1.61; δCH_3 0.89
Asp 72	118.6	8.30	4.66	2.86, 2.90	
Ala 73	123.7	8.13	4.31	1.41	
Arg 74	118.8	8.21	4.34	1.83, 1.95	γCH_2 1.68; δCH_2 3.22; εNH 7.25
Gly 75	109.0	8.30	3.98		
Gly 76	108.2	8.22	3.97		
Leu 77	120.7	8.07	4.38	1.62	γH 1.58; δCH_3 0.86, 0.91
Asp 78	120.6	8.47	4.96	2.73, 2.93	
Pro 79			4.41	2.03, 2.29	γCH_2 1.97, 2.02; δCH_2 3.80
Leu 80	120.1	8.10	4.32	1.68	γH 1.61; δCH_3 0.89, 0.94
Leu 81	122.1	7.93	4.38	1.66	γH 1.64; δCH_3 0.89, 0.96
Lys 82	124.3	8.03	4.28	1.77, 1.88	γCH_2 1.43; δCH_2 1.70; εCH_2 3.01

Side chain protons for which the chemical shifts deviate by more than 0.1 ppm from the random coil values (Bundi and Wüthrich 1979; Wüthrich 1986) are identified by underlining. Residues with αH chemical shifts deviating significantly from random coil values are identified in Fig. 3

References

Alexandrescu AT, Abeyguanwardana C, Shortle D (1994) Structure and dynamics of a denatured 131-residue fragment of staphylococcal nuclease: a heteronuclear NMR study. Biochemistry 33: 1063–1072

Åslund F, Ehn B, Miranda-Vizuete A, Pueyo C, Holmgren A (1994) Two additional glutaredoxins exist in Escherichia coli: glutaredoxin 3 is a hydrogen donor for ribonucleotide reductase in a thioredoxin/glutaredoxin 1 double mutant. Proc Natl Acad Sci, USA 91: 9813–9817

Åslund F, Nordstrand K, Berndt KD, Nikkola M, Bergman T, Postingl H, Jörnvall H, Otting G, Holmgren A (1996) Glutaredoxin-3 from Escherichia coli: amino acid sequence determination and structural analysis. J Biol Chem (in press)

Briand J, Ernst RR (1991) Computer-optimized homonuclear TOCSY experiments with suppression of cross relaxation. Chem Phys Lett 185: 276–285

Brüschweiler R, Griesinger C, Sørensen OW, Ernst RR (1988) Combined use of hard and soft pulses for ω_1 decoupling in two-dimensional NMR spectroscopy. J Magn Reson 78: 178–185

Bundi A, Wüthrich K (1979) ^{1}H-NMR parameters of the common amino acid residues measured in aqueous solutions of the linear tetrapeptides H-Gly-Gly-X-L-Ala-OH. Biopolymers 18: 285–297

Chou PY, Fasman GD (1974) Conformational parameters for amino acids in helical, beta-sheet, and random coil regions calculated from proteins. Biochemistry 13: 211–222

Logan TM, Thériault Y, Fesik SW (1994) Structural characterization of the FK506 binding protein unfolded in urea and guanidine hydrochloride. J Mol Biol 236: 637–648

Marion D, Ikura M, Tschudin R, Bax A (1989) Rapid recording of 2D NMR spectra without phase cycling. Application to the study of hydrogen exchange in proteins. J Magn Reson 85: 393–399

Messerle BA, Wider G, Otting G, Weber C, Wüthrich K (1989) Solvent suppression using a spin lock in 2D and 3D NMR spectroscopy with H_2O solutions. J Magn Reson 85: 608–613

Neri D, Wider G, Wüthrich K (1992a) Complete ^{15}N and ^{1}H NMR assignments for the amino-terminal domain of the phage 434 repressor in the urea-unfolded form. Proc Natl Acad Sci, USA 89: 4397–4401

Neri D, Wider G, Wüthrich K (1992b) ^{1}H, ^{15}N and ^{13}C NMR assignments of the 434 repressor fragments 1–63 and 44–63 unfolded in 7 M urea. FEBS Lett 303: 129–135

Otting G, Orbons LPM, Wüthrich K (1990) Suppression of zero-quantum coherence in NOESY and soft NOESY. J Magn Reson 89: 423–430

Stockman BJ, Euvrard A, Scahill TA (1993) Heteronuclear three-dimensional NMR spectroscopy of a partially denatured protein: the A-state of human ubiquitin. J Biomol NMR 3: 285–296

Szyperski T, Güntert P, Otting G, Wüthrich K (1992) Determination of scalar coupling constants by inverse Fourier transformation of in-phase multiplets. J Magn Reson 99: 552–560

Wishart DS, Sykes BD, Richards FM (1992) The chemical shift index: a fast and simple method for the assignment of protein secondary structure through NMR spectroscopy. Biochemistry 31: 1647–1651

Wüthrich K (1986) NMR of proteins and nucleic acids. Wiley, New York

Wüthrich K (1994) NMR assignments as a basis for structural characterization of denatured states of globular proteins. Curr Opin Struct Biol 4: 93–99

Zhang O, Kay LE, Olivier JP, Forman-Kay JD (1994) Backbone ^{1}H and ^{15}N resonance assignments of the N-terminal SH3 domain of drk in folded and unfolded states using enhanced-sensitivity pulsed field gradient NMR techniques. J Biomol NMR 4: 845–858