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Nuclear magnetic resonance study of the globular domain of chicken histone H5: Resonance assignment and secondary structure

(nuclear Overhauser effect/sequential resonance assignment)

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ABSTRACT **A ^{1}H NMR study of the 79-residue globular domain of chicken erythrocyte histone H5 (GH5) is presented. Using a combination of two-dimensional NMR techniques to demonstrate through-bond and through-space (<5 Å) connectivities, the resonances of GH5 are assigned in a sequential manner. From a qualitative interpretation of the short-range nuclear Overhauser effects (NOEs) involving the NH and C$^\alpha$H protons, it is shown that GH5 has four α-helices. The approximate spatial relationship of three of these four helices relative to each other is deduced from the observation of a number of long-range NOEs. The peptide chain outside the helices appears to have little regular secondary structure and no NOEs characteristic of β-sheets are apparent.**

Histones H1 and H5, the latter partially replacing the former in nucleated erythrocytes of birds, reptiles, and fish, are lysine-rich chromosomal proteins involved in the generation, maintenance, and control of higher-order chromatin structure (1). Both histones resemble each other in their primary structure, and CD, NMR, and hydrodynamic studies have shown that they are composed of a central globular core of ≈80 residues and disordered N- and C-terminal regions (2, 3). The globular domain, which can be obtained by treatment with trypsin, is able to close two full turns in the nucleosome and to protect from nuclease digestion an extra ≈20 base pairs of DNA present in the chromatosome above that in the core particle (4). At the present time, little is known about either the secondary or tertiary structure of the globular domain and to date it has not proved possible to obtain high-quality crystals diffracting to better than 5-Å resolution for x-ray crystallographic studies (S. Neidle, personal communication). An alternative approach involves the application of NMR spectroscopy in solution (5) and, in particular, the nuclear Overhauser effect (NOE) to obtain a large set of approximate interproton distances that can then be employed to determine the three-dimensional structure of the protein using either distance geometry algorithms (6, 7) or restrained molecular dynamics (8). As a first step toward this goal we present a ^{1}H NMR study of the 79-residue globular domain of chicken histone H5, known as GH5. Using a combination of two-dimensional NMR methods we first assign the resonances of GH5 in a sequential manner and then delineate secondary structure elements by means of a qualitative interpretation of the NOE data.

EXPERIMENTAL

GH5 was a gift of J. Gunning and S. Neidle. It was prepared by tryptic digestion of chicken erythrocyte histone H5 and purified as described (3). Samples for NMR contained 7 mM GH5 in either 90% H_2O/10% ^2H_2O or 99.96% ^2H_2O buffer consisting of 500 mM KCl, 50 mM phosphate (pH 3.7), and 0.1 mM EDTA.

All NMR measurements were carried out at 25°C on a Bruker AM 500 spectrometer. The following spectra were recorded: in ^2H_2O, an absolute-value homonuclear correlated (COSY) spectrum (9) and pure phase-absorption double-quantum-filtered COSY (DQF-COSY) (10), homonuclear Hartmann–Hahn (HOHAHA) spectra (11), and two-dimensional NOE (NOESY) spectra (12); in H_2O, absolute-value COSY and triple-quantum-filtered COSY (TQF-COSY) (13) spectra and pure phase-absorption HOHAHA and NOESY spectra. The HOHAHA spectra were recorded at several mixing times ranging from 15 to 80 ms in order to demonstrate successively direct, single and multiple relayed through-bond magnetization transfer (11). The NOESY spectra were recorded at mixing times of 100, 200, and 300 ms. Pure phase-absorption spectra were obtained by using the time proportional incrementation method (14). For measurements in H_2O, the H_2O resonance was suppressed by selective irradiation during the relaxation delay and, in the case of the NOESY spectra, during the mixing time as well. An additional set of NOESY spectra in H_2O was also recorded without solvent irradiation by replacing the last 90° pulse in the sequence by a semiselective jump–return (90_x-t-90_{-x}) pulse with the carrier placed at the position of the solvent (15).

RESULTS AND DISCUSSION

Resonance Assignment. Sequence-specific resonance assignments were obtained by first identifying amino acid spin systems by means of through-bond connectivities, followed by the sequential assignment of resonances by means of short (<5 Å) through-space connectivities (5, 16, 17, 22). The amino acid spin systems were principally identified using pure phase-absorption HOHAHA spectra recorded at several mixing times in order to obtain a series of spectra exhibiting either direct or direct and relayed (single and multiple) through-bond connectivities (11). Examples of such spectra are illustrated in Figs. 1 and 2 for the fingerprint NH-C$^\alpha$H region and for part of the aliphatic region showing connections from the C$^\alpha$H resonances to other aliphatic resonances, respectively. In this respect it is worth noting that all of the peaks in the HOHAHA spectra are in near absorption mode, in contrast to those in a COSY or DQF-COSY spectrum in which the individual multiplet components of the cross peaks are 180° out of phase relative to one another (18). This results not only in greater sensitivity but, more importantly, in narrower and sharper cross peaks. The increased resolution arising from the latter is particularly

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Abbreviations: NOE, nuclear Overhauser effect; NOESY, two-dimensional NOE; COSY, homonuclear correlated; DQF-COSY, double-quantum-filtered COSY; TQF-COSY, triple-quantum-filtered COSY; HOHAHA, homonuclear Hartmann–Hahn; GH5, globular domain of histone H5.

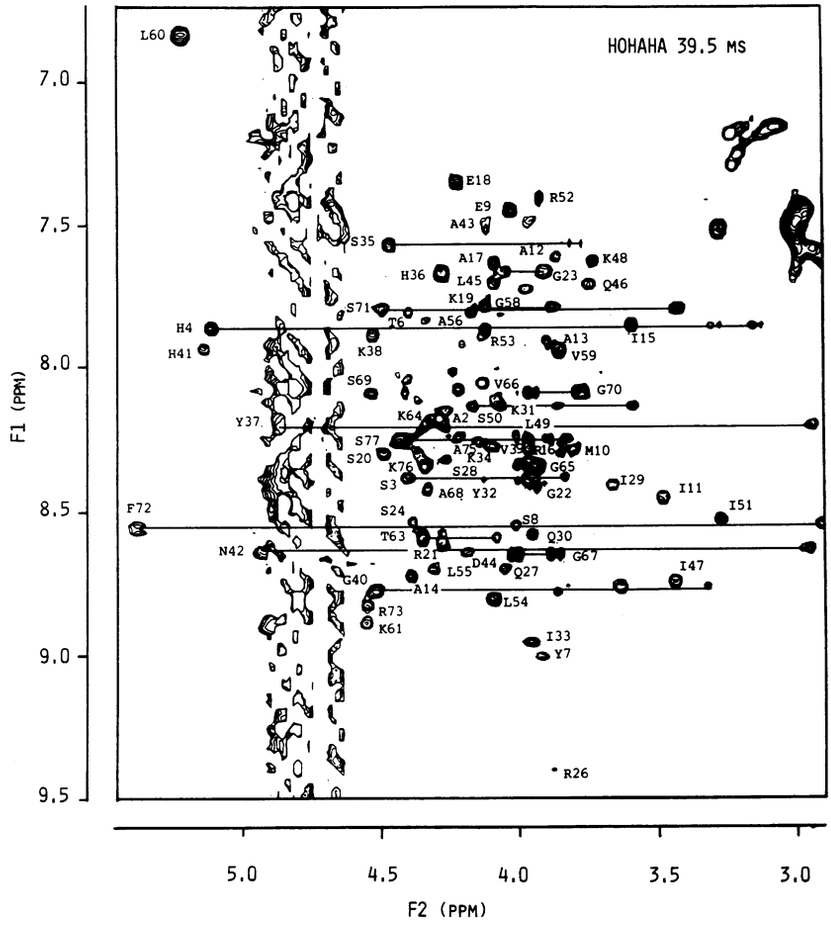

FIG. 1. NH (F1 axis)-C$^{\alpha}$H (F2 axis) region of the HOHAHA spectrum of GH5 in H_2O. Some C$^{\beta}$H resonances are also present in this region and relayed connectivities to the C$^{\beta}$H protons are indicated by continous lines.

useful for a system such as the present one in which the chemical shift dispersion of the resonances is limited. Nevertheless, the COSY and DQF-COSY spectra were quite helpful for the purpose of distinguishing direct from relayed connectivities in the HOHAHA spectra in a few ambiguous instances. In addition, the TQF-COSY spectrum in H_2O proved useful in identifying six of the seven glycine spin systems (13).

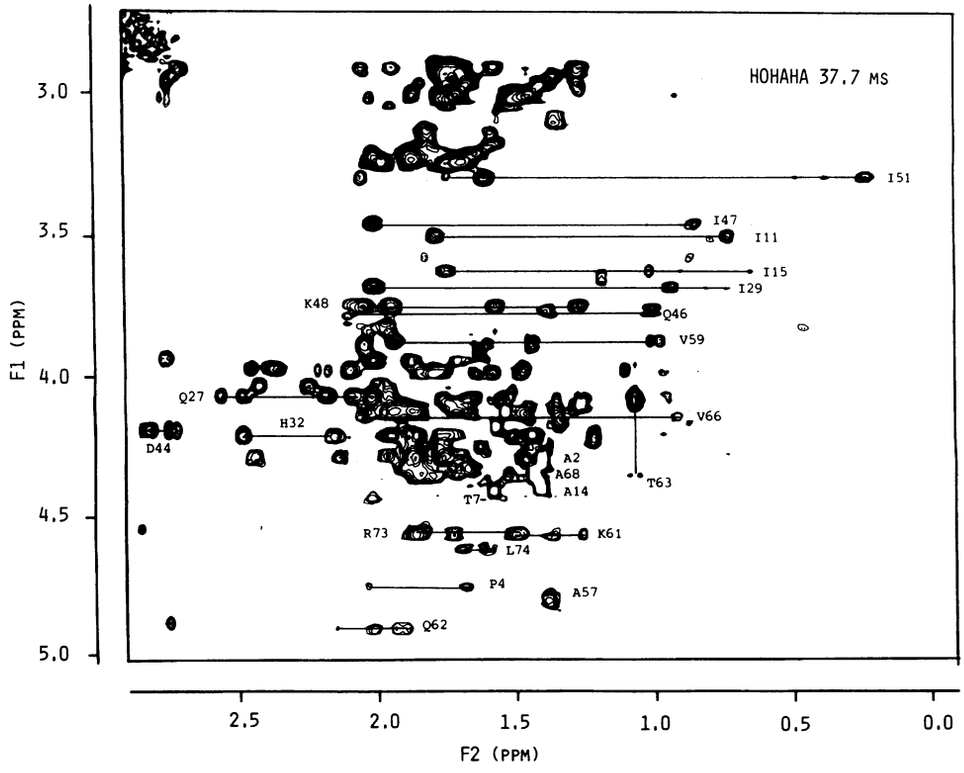

FIG. 2. Portion of the C$^{\alpha}$H (F1 axis)-aliphatic (F2 axis) region of the HOHAHA spectrum in ^2H_2O. Direct and relayed connectivities are present and some spin systems are indicated by continuous lines.

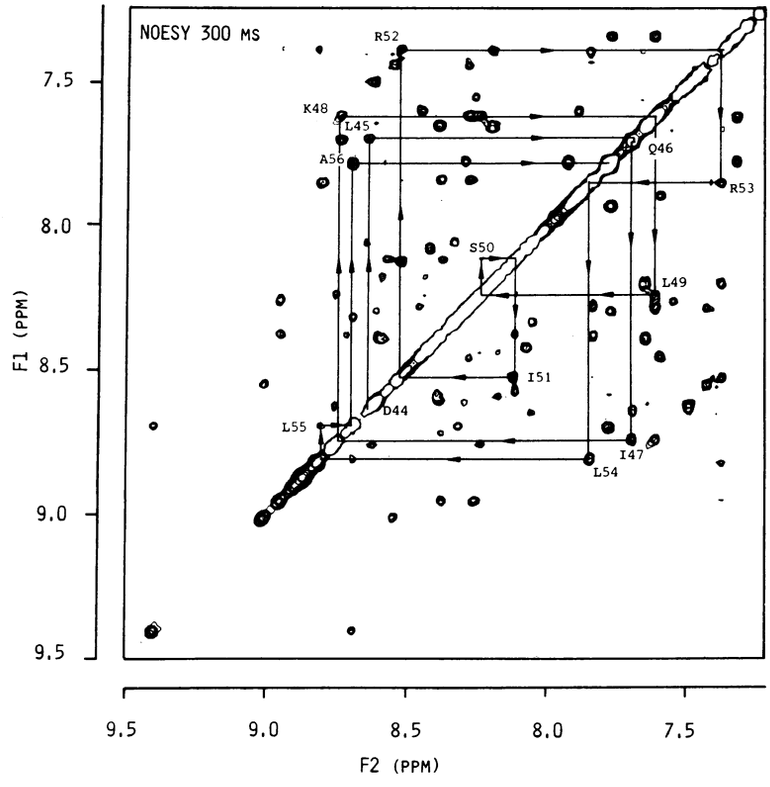

FIG. 3. Portion of the NH (F1 axis)-NH (F2 axis) region of the NOESY spectrum of GH5 in H_2O. The sequence of $d_{NN}(i, i + 1)$ connectivities extending from Asn-44 to Ala-56, constituting helix IV, is indicated by continuous lines.

For sequential assignment the interresidue $C^{\alpha}H(i)$-NH(i + 1) ($d_{\alpha N}$), NH(i)-NH(i + 1) (d_{NN}), and $C^{\beta}H(i)$-NH_{i+1} ($d_{\beta N}$) through-space connectivities are the most important (5). Some examples of these are shown in Figs. 3 and 4 for the NH-NH and NH-$C^{\alpha}H$ regions of the pure phase-absorption NOESY spectra. The short-range NOEs involving the NH, $C^{\alpha}H$, and $C^{\beta}H$ protons formed a continuous set of sequential through-space connectivities along the polypeptide chain with the exception of eight breaks—in particular, between His-4 and Thr-6, Ser-24 and Ser-25, Tyr-37 and Lys-38, Val-39 and His-41, Ala-57 and Gly-58, Lys-61 and Gln-62, Ser-69 and Gly-70, and Ala-73 and Leu-74. A summary of the observed short-range NOEs involving the NH, $C^{\alpha}H$, and $C^{\beta}H$ protons is given in Fig. 5 and the assignments of the proton resonances are listed in Table 1.

Secondary Structure. From a detailed analysis of short-range interproton distances of <4 Å involving NH, $C^{\alpha}H$, and $C^{\beta}H$ protons present in various protein structure elements, it has been shown that it is possible to delineate with reasonable accuracy regular secondary structure elements of a polypeptide from a qualitative analysis of the NOE data (20, 21). This is because the magnitudes of the NOE cross peaks at short mixing times are approximately proportional to r^{-6} and therefore very sensitive to the value of the interproton distance r. At the same time, each type of secondary structure element has a particular set of short interproton distances associated with it and hence exhibits a characteristic pattern of NOEs.

α-Helices are characterized by a continuous stretch of strong $d_{NN}(i, i + 1)$ NOEs, medium $d_{\alpha N}(i, i + 3)$ and $d_{\alpha\beta}(i, i + 3)$ NOEs, and weak or absent $d_{\alpha N}(i, i + 1)$ NOEs. [Note that $d_{\beta N}(i, i + 1)$ NOEs are not structurally useful as they show little dependence on secondary structure type.] Because of extensive resonance overlap we were unable to assign unambiguously $d_{\alpha\beta}(i, i + 3)$ cross peaks. Nevertheless, the remaining connectivities involving the NH and $C^{\alpha}H$ protons are sufficient to permit us to delineate four α-helical

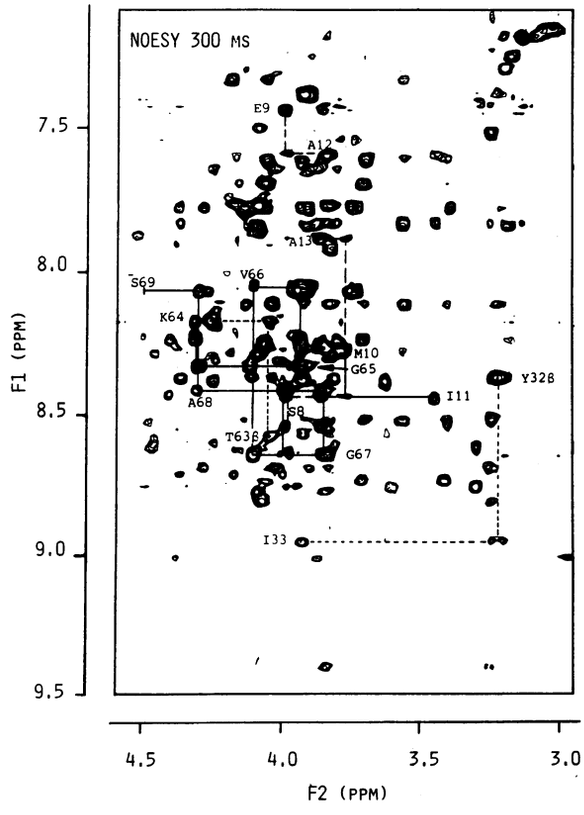

FIG. 4. Portion of the NH (F1 axis)-$C^{\alpha}H/C^{\beta}H$ (F2 axis) region of the NOESY spectrum of GH5 in H_2O. Some $d_{\alpha N}(i, i + 1)$, $d_{\alpha N}(i, i + 3)$, and $d_{\beta N}(i, i + 1)$ connectivities are indicated by the symbols —, – – –, and ----, respectively. Peaks are labeled at the position of the NH(i)-$C^{\alpha}H(i)$ and NH(i)-$C^{\beta}H(i)$ intraresidue cross peaks. The latter are indicated by the symbol β.

Table 1. Proton resonance assignments of GH5 at 25°C

Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	Other	Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	Other
S1	NA				H41	7.91	5.11	3.34, 3.20	$C^{\delta}H$ 8.62; $C^{\epsilon}H$ 7.44
A2	8.17	4.26	1.38		N42	8.62	4.93	2.97	
S3	8.37	4.37	3.81		A43	7.50	4.09	1.52	
H4	7.84	5.08	3.24, 3.14	$C^{\delta}H$ 8.62; $C^{\epsilon}H$ 7.44	D44	8.58	4.15	2.93, 2.84	
P5		4.75	2.40, 1.63	$C^{\gamma}H$ 2.01, 1.84; $C^{\delta}H$ 3.84, 3.38	L45	7.69	4.07	1.74	
T6	7.79	4.39	1.55	$C^{\gamma}H_3$ 1.55	Q46	7.70	3.72	1.38, 0.99	$C^{\gamma}H$ 2.09
Y7	9.01	3.90	3.02, 2.74	$C^{\delta}H$ 7.06; $C^{\epsilon}H$ 6.67	I47	8.74	3.42	1.99	$C^{\gamma}H_3$ 0.85; $C^{\gamma}H$ 1.02
S8	8.55	4.01	3.86		K48	7.62	3.70	2.03, 1.92	$C^{\gamma}H$ 1.26; $C^{\delta}H$ 1.55
E9	7.45	4.04	2.41, 2.24	$C^{\gamma}H$ 1.97	L49	8.24	3.94	1.72, 1.46	$C^{\gamma}H$ 1.64
M10	8.29	3.78	1.96, 1.55	$C^{\gamma}H$ 2.11, 1.36	S50	8.12	4.15	3.86, 3.59	
I11	8.44	3.46	1.77	$C^{\gamma}H_3$ 0.72	I51	8.47	3.24	1.71	$C^{\gamma}H_3$ 0.23; $C^{\gamma}H$ 0.48
A12	7.62	3.84	1.42		R52	7.38	3.90	1.99	$C^{\gamma}H$ 1.85, 1.68; $C^{\delta}H$ 3.21; $N^{\epsilon}H$ 7.24
A13	7.89	3.89	1.61		R53	7.85	4.09	2.01, 1.95	$C^{\gamma}H$ 1.82, 1.67; $C^{\delta}H$ 3.23; $N^{\epsilon}H$ 7.31
A14	8.72	4.37	1.39		L54	8.82	4.08	1.91	$C^{\gamma}H$ 1.28; $C^{\delta}H_3$ 0.75
I15	7.84	3.57	1.71	$C^{\gamma}H_3$ 1.01; $C^{\gamma}H$ 0.64; $C^{\delta}H_3$ 0.89	L55	8.79	4.29	1.88, 1.65	
R16	8.28	3.97	1.80	$C^{\gamma}H$ 1.57	A56	7.78	4.14	1.55	
A17	7.64	4.08	1.24		A57	8.53	4.78	1.34	
E18	7.33	4.19	2.48, 1.92	$C^{\gamma}H$ 2.14	G58	7.78	4.12, 3.86		
K19	7.77	4.09	1.93		V59	7.93	3.83	1.91	$C^{\gamma}H_3$ 0.99, 0.95
S20	8.29	4.45	3.84		L60	6.81	5.22	1.26	$C^{\gamma}H$ 1.53; $C^{\delta}H_3$ 0.67
R21	8.61	4.25	1.95, 1.85	$C^{\gamma}H$ 1.73; $C^{\delta}H$ 3.24	K61	8.89	4.54	1.49	$C^{\gamma}H$ 1.25
G22	8.39	3.93			Q62	8.88	4.90	2.01	$C^{\gamma}H$ 2.14
G23	7.65	4.03, 3.88			T63	8.58	4.32	4.06	$C^{\gamma}H_3$ 1.04
S24	8.53	4.36	3.98		K64	8.20	4.32	1.84, 1.73	
S25	8.91	4.94	3.86, 3.63		G65	8.33	4.03		
R26	9.41	3.87	2.04	$C^{\gamma}H$ 1.58; $C^{\delta}H$ 3.28; $N^{\epsilon}H$ 7.53	V66	8.05	4.11	2.04	$C^{\gamma}H_3$ 0.91
Q27	8.69	4.02	2.17, 2.08	$C^{\gamma}H$ 2.56, 2.48	G67	8.64	3.99, 3.84		
S28	8.32	4.24	4.16, 4.05		A68	8.43	4.31	1.41	
I29	8.40	3.64	1.99	$C^{\gamma}H_3$ 0.93	S69	8.07	4.52	3.98	
Q30	8.58	3.95	2.16	$C^{\gamma}H$ 2.42, 2.33	G70	8.07	4.03	3.75	
K31	8.12	4.04	2.01	$C^{\gamma}H$ 1.51; $C^{\delta}H$ 1.75; $C^{\epsilon}H$ 3.20	S71	7.78	4.47	3.41	
Y32	8.39	4.10	3.24	$C^{\delta}H$ 6.93; $C^{\epsilon}H$ 6.42	F72	8.53	5.38	2.91, 2.69	$C^{\delta}H$ 7.04; $C^{\epsilon}H$ 7.18; $C^{\zeta}H$ 7.26
I33	8.96	3.94	2.33	$C^{\gamma}H_3$ 0.83; $C^{\gamma}H$ 1.13	R73	8.83	4.54	1.85	$C^{\gamma}H$ 1.49
K34	8.24	4.07	1.65		L74	8.64	4.61	1.67, 1.59	
S35	7.56	4.44	3.84, 3.78		A75	7.25	4.13	1.30	
H36	7.65	4.27	2.42, 2.12	$C^{\delta}H$ 8.23; $C^{\epsilon}H$ 6.85	K76	8.34	4.31	1.76	
Y37	8.22	4.88	2.96, 2.74	$C^{\delta}H$ 7.40; $C^{\epsilon}H$ 6.81	S77	7.24	4.40	3.87, 3.81	
K38	7.97	4.50	0.88		D78	8.43	4.67	2.86, 2.77	
V39	8.28	3.94	2.09	$C^{\gamma}H_3$ 1.09, 0.95	K79	NA			
G40	NA								

Chemical shifts are in ppm relative to 4,4-dimethylsilapentane-1-sulfonate. NA, not assigned.

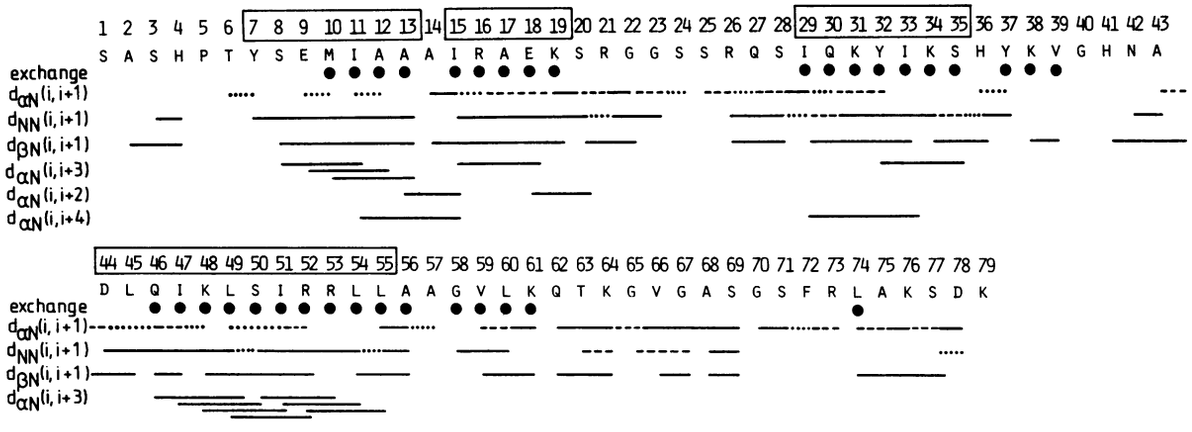

FIG. 5. Sequence of GH5 (3, 19) together with a summary of the observed short-range interresidue NOEs involving the NH, $C^{\alpha}H$, and $C^{\beta}H$ resonances. The $d_{NN}(i, i+1)$ and $d_{\alpha N}(i, i+1)$ NOEs are classified into strong (—), medium (----), and weak (·····). Intensities of the other NOEs are not classified. Very slowly exchanging (>24 hr) NH protons are indicated by closed circles and the helices are indicated by boxes.

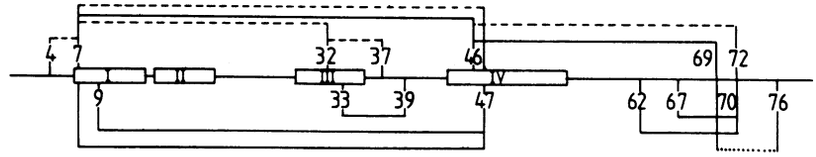

FIG. 6. Summary of the long-range NOEs. ---, Side chain-side chain NOEs; —, side chain-backbone NOEs; and ·····, backbone-backbone NOEs. (The NOEs involving the side chain protons include the $C^{\beta}H$ protons.)

regions (Fig. 5) extending from residues 7 to 13 (helix I), 15 to 19 (helix II), 29 to 35 (helix III), and 44 to 55 (helix IV). This is also supported by the observation of very slowly exchanging (i.e., >24 hr) amide protons in these regions (22). In the region from residues 13 to 15 the following pattern of NOEs is observed: no d_{NN} NOEs, a strong $d_{\alpha N}(i, i + 1)$ NOE between residues 14 and 15, a $d_{\alpha N}(i, i + 2)$ NOE from residues 13 to 15, and a $d_{\alpha N}(i, i + 4)$ NOE from residues 11 to 15. These NOEs are indicative of a change in orientation of helix II with respect to helix I, associated with a change in the ϕ angle of residue 14 from the $-40°$ to $-70°$ range characteristic of a helix to the $-180° \pm 60°$ range. β strands, on the other hand, are characterized by a stretch of very strong $d_{\alpha N}(i, i + 1)$ NOEs and the absence of the other short-range NOEs involving the NH and $C^{\alpha}H$ protons. The only regions in which this appears to be the case are residues 71 to 73 and 74 to 77. Further, no evidence for parallel or anti-parallel β sheets in terms of interstrand $C^{\alpha}H$-$C^{\alpha}H$ and NH-NH connectivities could be obtained. All other regions appear to have irregular secondary structures with the exception of the regions from residues 24 to 27, which have a pattern of $d_{NN}(i, i + 1)$ and $d_{\alpha N}(i, i + 1)$ NOEs characteristic of either a half-turn or a type II turn (23).

Spatial Relationship of the Helices. In addition to short-range NOEs, a number of long-range NOEs involving backbone and side chain protons have been identified to date. These are summarized in Fig. 6. Of particular interest are the NOEs from Tyr-7 of helix I to Tyr-32 of helix III, Gln-46 of helix IV and Ile-47 of helix IV, and from Glu-9 of helix I to Ile-47 of helix IV. These NOEs indicate that the N-terminal ends of helices I and IV and the central region of helix III must be close to each other in space. Inspection of Fig. 6 also reveals a number of other interesting features. The NOE between the side chains of His-4 and Tyr-7 suggests the occurrence of a turn involving Pro-5. The NOEs between Tyr-32 and Tyr-37 and between Ile-33 and Val-39 indicate a change in direction of the peptide chain following helix III, and model building suggests that the angle between the long axes of helices III and IV is in the region of 40° to 80°. A series of loops are also indicated by the NOEs from Glu-62 and Gly-67 to Phe-72 and from Ser-69 or Gly-70 to Lys-76. Further, the segment from residues 62 to 79 must be close to the N-terminal end of helix IV on account of the NOEs from Gln-46 to either Ser-69 or Gly-70 and Phe-72. (Note that the NH protons of Ser-69 and Gly-70 have the same chemical shift so that NOEs involving this position such as those described above cannot be assigned to one or the other residue unambiguously.) Many of the long range NOEs involve nonpolar side chains and include all of the aromatic residues. These NOEs are indicative of a hydrophobic core formed by the intersection of helices I, III, and IV and a segment of the C-terminal peptide chain comprising Phe-72.

Further detailed analysis of the long-range NOE connectivities must be carried out to arrive at a more complete picture of the three-dimensional structure of GH5.

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