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A method for the estimation of χ^1 torsion angles in proteins

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SUMMARY

A method for estimating αCH–βCH coupling constants from the shape and fine structure of NH–αCH fingerprint-region cross peaks of COSY spectra is presented. Spectral simulations have been used to analyse the effect of variations in $^3J_{NH-\alpha CH}$, $^3J_{\alpha CH-\beta CH}$, linewidths and digital resolution on the appearance of NH–αCH COSY cross peaks. On the basis of these simulations a set of rules for broadly categorising experimental NH–αCH cross peaks according to αCH–βCH coupling constants has been devised. The method has been applied to the analysis of NH–αCH cross peaks of hen lysozyme. The results are compared to previous measurements of αCH–βCH coupling constants using E.COSY techniques.

INTRODUCTION

Protein structures in solution are based on a large number of distance restraints derived from nuclear Overhauser enhancement (NOE) effects and on torsion-angle restraints derived from measured spin–spin coupling constants (Wüthrich, 1986). Restraints for the torsion angle χ^1 are based on measured $^3J_{\alpha CH-\beta CH}$ values and qualitative rather than quantitative information about these coupling constants is usually sufficient. For example, two measured αCH–βCH coupling constants of < 5 Hz correspond to $\chi^1 = +60°$, whereas one large coupling constant (> 10 Hz) and one small coupling constant (< 5 Hz) correspond to $\chi^1 = -60°$ or $\chi^1 = 180°$; additional information about the relative intensities of the NH–βCH and αCH–βCH NOE effects is needed to distinguish between $\chi^1 = -60°$ and $\chi^1 = 180°$ (Wagner et al., 1987; Arseniev et al., 1988). Pairs of αCH–βCH coupling constants which differ by less than 5 Hz usually indicate side-chain averaging about χ^1 and are not, therefore, interpreted in terms of χ^1 angle restraints.

αCH–βCH coupling constants, for residues with two β-protons, can be obtained from the relative displacements of cross-peak components in E.COSY and P.E.COSY spectra recorded in

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D_2O (Griesinger et al., 1987; Mueller, 1987). For threonine, isoleucine and valine residues, which contain only one β-proton, the αCH–βCH coupling constant must be measured from the antiphase splitting through αCH–βCH cross peaks of COSY spectra collected in D_2O and corrections for the distortions due to linewidth must be taken into account (Smith et al., 1991). Both of these methods for measuring αCH–βCH coupling constants suffer from limitations. The αCH–βCH region of E.COSY spectra is often very crowded and overlap of cross peaks can make accurate measurements of coupling constants impossible. Cross-peak intensity in COSY or E.COSY spectra can be very weak if the active αCH–βCH coupling constant is small; poor signal-to-noise ratios result in errors in the measured αCH–βCH coupling constants. The measurement of coupling constants is particularly difficult in the case where $\chi^1 = +60°$ because both αCH–βCH cross peaks will be of low intensity.

In this paper an alternative method for the estimation of αCH–βCH coupling constants is presented. This method is based on analysis of the NH–αCH cross peak rather than the αCH–βCH cross peak. Use of the fingerprint region of the spectrum has the advantage of the good chemical-shift dispersion usually observed for the NH resonances. The analysis of the NH–αCH cross peaks presented here provides qualitative information about the αCH–βCH coupling constants which is useful for determining χ^1 angle restraints for structure calculations, for obtaining stereospecific assignments for βCH resonances in conjunction with NOE data, and for making assignments of cross peaks in the αCH–βCH region of the COSY spectrum. The method has been applied to the estimation of χ^1 values for hen egg-white lysozyme.

METHODS

Hen egg-white lysozyme was obtained from Sigma Chemical Company and dialysed at pH 3 to remove acetate. The protein was dissolved in 90% H_2O/10% D_2O at a concentration of 7 mM and at pH 3.8. Phase-sensitive COSY spectra were recorded at 55 °C on a home-built 500 MHz NMR spectrometer of the Oxford Centre for Molecular Sciences. The spectrometer is equipped with an Oxford Instrument Company magnet, a GE/Nicolet 1280 data acquisition system, a 293D pulse programmer and a Bruker probe. Data were processed using FTNMR and FELIX software (Hare Research Inc.) running on SUN computers of the Oxford Centre for Molecular Sciences. Standard phase-cycling schemes were used (Aue et al., 1976; Bax and Freeman, 1981). Phase discrimination in t_1 was achieved using the method of States et al. (1982). The transmitter frequency was set at the frequency of the water resonance and this resonance was suppressed by low-power, continuous wave irradiation during a 1-s preparation delay. 512 complex t_1 increments of 4096 complex points were collected. 32 transients were collected for each t_1 increment. Spectral widths of 5405 Hz were used. The spectra were resolution enhanced prior to Fourier transformation by double exponential multiplication (DM = 3.75) and trapezoidal multiplication (T1 = 256, T2 = 0) in the F_2 dimension and by trapezoidal multiplication (T1 = T2 = 128) in the F_1 dimension. After zero-filling once in the F_2 dimension and twice in the F_1 dimension the digital resolution was 0.66 Hz/point in F_2 and 2.6 Hz/point in F_1. The horizontal axis is the F_2 dimension and the vertical axis is the F_1 dimension.

Peak-shape information was extracted from the experimental and simulated cross peaks as follows. A cross peak is selected in the spectrum. The contour scaling factor is set equal to the average peak height of the four antiphase components and the cross peak is plotted with four

contour levels. The contours are spaced logarithmically so that the n^{th} level corresponds to an intensity of 0.7^n times the scaling factor; the four levels are drawn at 0.7, 0.49, 0.34 and 0.24 times the scaling factor. The peak shape is assessed quantitatively on the basis of two ratios, R_1 and R_2, derived from the measurement of m, n, p and q as shown in Fig. 1a (R_1 = m/n, R_2 = p/q). The positions of the peak centres, required for the measurement of m and n, are interpolated by eye. The values of p and q are taken from the lowest plotted contour (corresponding to 0.24 times the

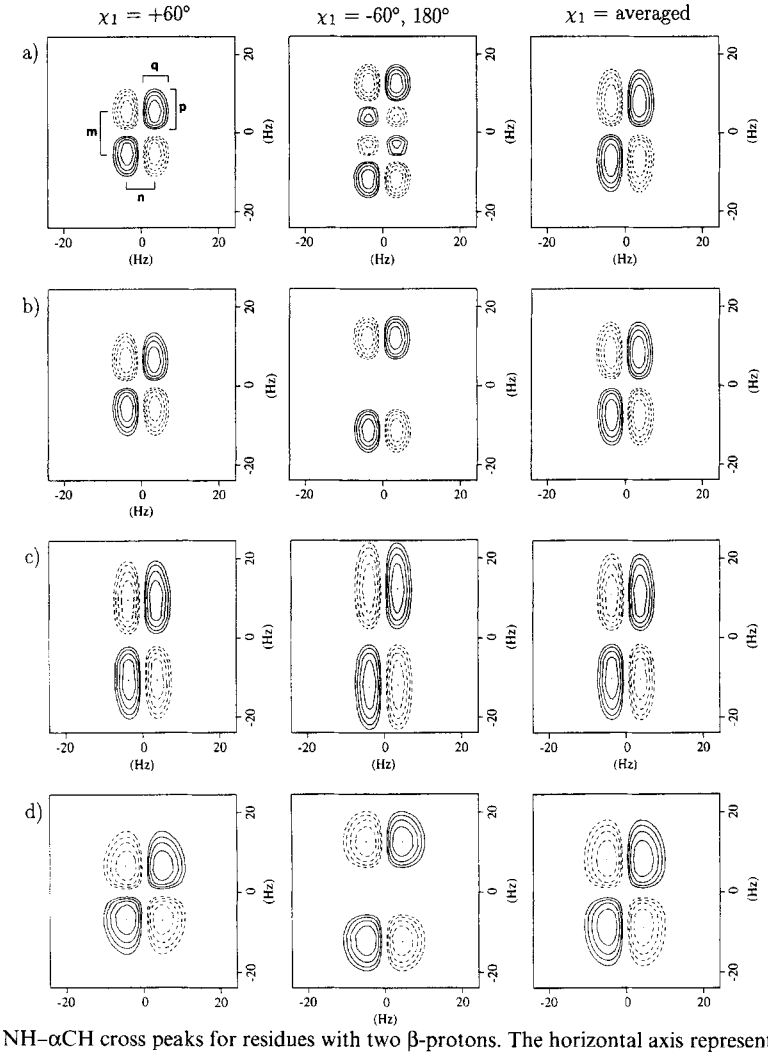

Fig. 1. Simulated NH–αCH cross peaks for residues with two β-protons. The horizontal axis represents F_2 (NH) and the vertical axis represents F_1 (αCH). All peaks were simulated with $^3J_{NH-\alpha CH}$ = 7.0 Hz. The peaks in row (a) were simulated with linewidths of 7.5 Hz in F_1 and 5.5 Hz in F_1, and with 512 t_1 values. The peaks in row (b) were simulated with linewidths of 7.5 Hz in F_2 and 5.5 Hz in F_1, and with 512 t_1 values; the χ^1 values were averaged by +/−30° about the stated value. The peaks in row (c) were simulated with linewidths of 7.5 Hz in F_2 and 5.5 Hz in F_1, and with 256 t_1 values. The peaks in row (d) were simulated with linewidths of 15.5 Hz in F_1 and F_2, and with 512 t_1 values. The two ratios R_1 and R_2, used to measure overall peak shape and individual peak component shape, are defined as shown in the upper left-hand square: R_1 = m/n, R_2 = p/q.

peak height). In the case of experimental peaks the values of p and q are measured for all four cross-peak components and the values of m and n are measured twice. The outer cross-peak components are used for the measurement of m, n, p and q in the case of cross peaks with eight components. This procedure is repeated for each cross peak using the scaling factor appropriate for that peak.

NH–αCH cross peaks were simulated by using the program SIMULATION (Redfield, unpublished data). The program assumes weak coupling and applies experimental parameters for digital resolution and resolution enhancement to antiphase cross peaks with Lorentzian lineshapes.

Coordinates for hen lysozyme were taken from the 2.0-Å resolution tetragonal type 2 crystal structure obtained by X-ray diffraction (Blake et al., 1967; Handoll, 1985) and from the 1.4-Å triclinic crystal structure obtained by neutron diffraction and provided by Dr. Sax Mason (Grenoble). Torsion angles are defined by the non-hydrogen atoms according to the IUPAC-IUB convention (1970). Coupling constants were computed using a Karplus equation (Karplus, 1959) with A, B and C values of 9.5, −1.6 and 1.8, respectively (DeMarco et al., 1978).

RESULTS AND DISCUSSION

A portion of the fingerprint region of the COSY spectrum of hen lysozyme is shown in Fig. 2a. Close inspection of this spectrum reveals that the NH–αCH cross peaks have a variety of shapes. For example, the peaks arising from D52 and Y53 have a pronounced rectangular shape whereas those arising from D66 and I78 have a squarer shape. This variation in cross-peak shape is very pronounced when 512 complex increments are collected in the t_1 dimension but is lost to a great extent when only 256 t_1 increments are collected as shown in Fig. 2b (Redfield, 1990). The cross-peak shape in the F_2 dimension is affected by the active NH–αCH coupling constant while the peak shape in F_1 is affected by both the active NH–αCH and passive αCH–βCH couplings. Thus, analysis of the fingerprint cross peaks may provide some information about the αCH–βCH coupling constants.

NH–αCH cross peaks have been simulated for various values of $^3J_{NH-\alpha CH}$ and $^3J_{\alpha CH-\beta CH}$ in order to understand the influence of these coupling constants on the observed NH–αCH cross-peak shapes; examples of these simulations are illustrated in Figs. 1 and 3. The overall peak shape and cross-peak component shape can be assessed quantitatively using the measurements shown in Fig. 1a; the overall peak shape is expressed by the ratio R_1 whereas the individual cross-peak component shape is expressed by R_2. The ratios R_1 and R_2, expected for various values of $^3J_{NH-\alpha CH}$ and $^3J_{\alpha CH-\beta CH}$, are summarised in Table 1. Several conclusions can be drawn from these simulations. The observed cross-peak shape is influenced strongly by the passive αCH–βCH coupling constants. A square peak shape is observed when the single αCH–βCH coupling or both αCH–βCH couplings are small. Thus, a square cross-peak shape is characteristic of a χ^1 angle close to 60° for residues with two β-protons, to a χ^1 angle close to 60° or 180° for threonines and isoleucines and to a χ^1 angle close to 60° or −60° for valines. This square shape is characterised by R_1 and R_2 values that are both less than 2.0. A rectangular cross peak is observed when one of the αCH–βCH coupling constants is large; that is, when the χ^1 angle is close to −60° or 180° for residues with two β-protons, close to −60° for threonines and isoleucines and close to 180° for valines. Rectangular cross peaks are often composed of eight components. However, the four

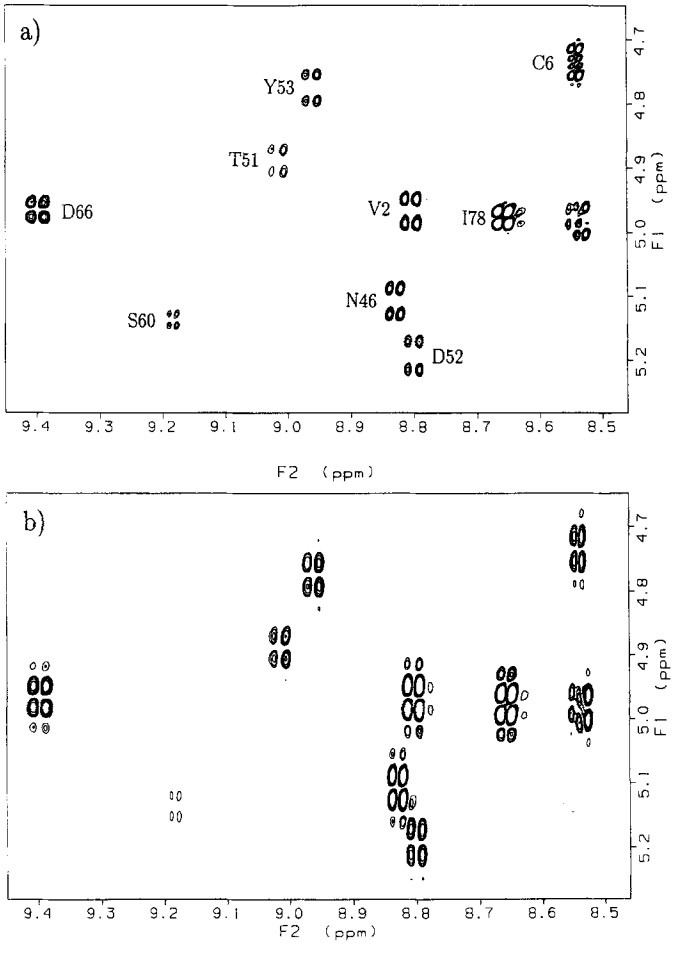

Fig. 2. Part of the fingerprint region of the phase-sensitive COSY spectrum of hen egg-white lysozyme collected with (a) 512 complex t_1 increments and (b) 256 complex t_1 increments.

central components may be of lower intensity than the outer components or altogether absent due to cancellation arising from a larger αCH linewidth or a small amount of averaging about χ^1. The rectangular shape is characterised by R_1 values of more than 2.0 and R_2 values of less than 2.0. Cross peaks with a shape between square and rectangular are classified as intermediate; this shape arises when the αCH–βCH coupling constants are between 5 and 10 Hz and is characteristic of extensive motional averaging about χ^1. The intermediate peaks can be distinguished from the square and rectangular ones on the basis of the shape of the individual cross-peak components. Cross-peak components have a more circular shape when the side chain is in a specific χ^1 conformation (60°, 180° or −60°), whereas the components are more elongated along F_1 when the coupling constants are averaged. The intermediate cross-peak shape is characterised by R_1 values between 1.5 and 3.0 and R_2 values greater than 2.0. Alanine residues, which contain three β-protons, give rise to NH–αCH cross peaks of intermediate shape similar to those obtained above for motional averaging about χ^1.

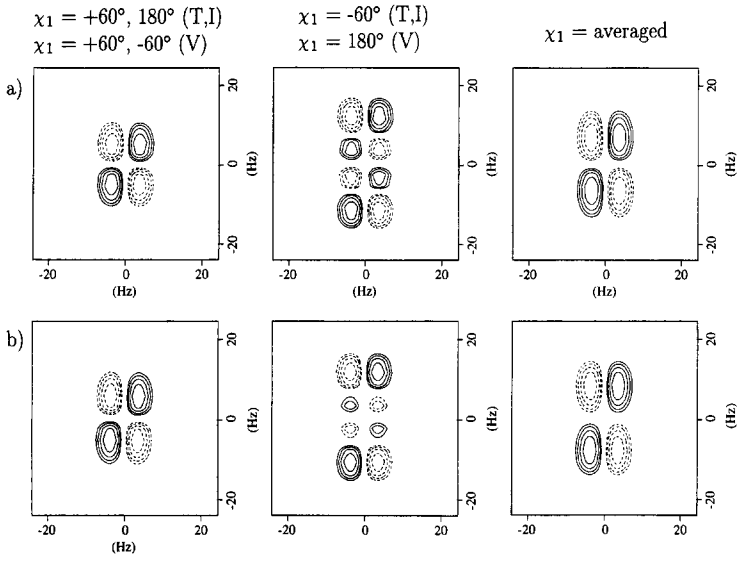

Fig. 3. Simulated NH–αCH cross peaks for residues with one β-proton. The horizontal axis represents F_2 (NH) and the vertical axis represents F_1 (αCH). All peaks were simulated with $^3J_{NH-\alpha CH}$ = 7.0 Hz. The peaks in row (a) were simulated with linewidths of 7.5 Hz in F_2 and 5.5 Hz in F_1, and with 512 t_1 values. The peaks in row (b) were simulated with linewidths of 7.5 Hz in F_2 and 5.5 Hz in F_1, and with 512 t_1 values; the χ^1 values were averaged by +/−30° about the stated value.

A close inspection of the R_1 values listed in Table 1 shows that these depend on the value of $^3J_{NH-\alpha CH}$ as well as on $^3J_{\alpha CH-\beta CH}$; the value of R_1 decreases as $^3J_{NH-\alpha CH}$ increases. This arises because the splitting in F_2 is more sensitive to changes in $^3J_{NH-\alpha CH}$ than the splitting in F_1. The ratio R_2 is fairly insensitive to $^3J_{NH-\alpha CH}$ for all cross-peak types. The extent to which cross-peak shape information, in the form of the ratios R_1 and R_2, can be used to obtain χ^1 information will depend on whether the value of the NH–αCH coupling constant is known. If $^3J_{NH-\alpha CH}$ has been measured then χ^1 information can always be obtained from R_1 and R_2. If $^3J_{NH-\alpha CH}$ is not known then ambiguities may arise. For example, an R_1 value of 2.5 is characteristic of a rectangular peak when $^3J_{NH-\alpha CH}$ is large but is characteristic of an intermediate peak if $^3J_{NH-\alpha CH}$ is small. However, an R_1 value greater than 4.0 is always characteristic of a rectangular peak and indicates that $^3J_{NH-\alpha CH}$ must be small. A set of rules for classifying peaks as square, rectangular or intermediate on the basis of the R_1 and R_2 values is summarised in Table 2.

The effects of linewidth and digital resolution in F_1 on the observed peak shapes have also been investigated using simulations. Cross peaks simulated on the basis of 256 complex t_1 increments no longer have the characteristic shapes obtained using 512 t_1 increments as shown in Fig. 1c. They all have R_1 and R_2 values characteristic of the intermediate shape and cannot be used to obtain χ^1 information. Increasing the values of the NH and αCH linewidths also leads eventually to a loss of peak-shape definition. However, even with linewidths of about 15 Hz useful information about χ^1 can be obtained from the NH–αCH peak shape.

The fingerprint region of the COSY spectrum of hen lysozyme has been assigned fully (Redfield and Dobson, 1988). NH–αCH coupling constants have been measured for the majority of residues of lysozyme and αCH–βCH coupling constants are known for 57 residues (Smith et al.,

TABLE 1
R_1 AND R_2 VALUES CALCULATED FOR VARIOUS TYPES OF NH–αCH CROSS PEAKS

	$J_{N\alpha}$ = 4 Hz		$J_{N\alpha}$ = 7 Hz		$J_{N\alpha}$ = 10 Hz	
	R_1	R_2	R_1	R_2	R_1	R_2
Two β-protons, 512 t_1 values, $LW(F_1)$ = 5.5 Hz, $LW(F_2)$ = 7.5 Hz						
χ^1 = −60°, 180°; $^3J_{\alpha\beta}$ = 3.4, 12.9 Hz	4.5	1.5	3.4	1.4	2.6	1.4
χ^1 = −60°, 180° +/−30°; $^3J_{\alpha\beta}$ = 5.0, 11.5 Hz	4.3	1.7	3.3	1.6	2.3	1.7
χ^1 = +60°; $^3J_{\alpha\beta}$ = 3.4, 3.4 Hz	1.9	1.7	1.6	1.6	1.3	1.6
χ^1 = +60°, +/−30°; $^3J_{\alpha\beta}$ = 5.0, 5.0 Hz	2.3	1.9	1.8	1.8	1.3	1.8
χ^1 = averaged; $^3J_{\alpha\beta}$ = 6.7, 6.7 Hz	2.5	2.5	2.1	2.2	1.6	2.1
χ^1 = averaged; $^3J_{\alpha\beta}$ = 5.7, 7.7 Hz	3.1	2.4	2.2	2.1	1.6	2.1

	$J_{N\alpha}$ = 7 Hz	
	R_1	R_2
Two β-protons, 256 t_1 values, $LW(F_1)$ = 5.5 Hz, $LW(F_2)$ = 7.5 Hz		
χ^1 = −60°, 180°; $^3J_{\alpha\beta}$ = 3.4, 12.9 Hz	3.3	3.3
χ^1 = +60°; $^3J_{\alpha\beta}$ = 3.4, 3.4 Hz	3.1	2.8
χ^1 = averaged; $^3J_{\alpha\beta}$ = 6.7, 6.7 Hz	2.9	2.8

	$J_{N\alpha}$ = 7 Hz	
	R_1	R_2
Two β-protons, 512 t_1 values, $LW(F_1)$ = 15.5 Hz, $LW(F_2)$ = 15.5 Hz		
χ^1 = −60°, 180°; $^3J_{\alpha\beta}$ = 3.4, 12.9 Hz	2.7	1.4
χ^1 = +60°; $^3J_{\alpha\beta}$ = 3.4, 3.4 Hz	1.4	1.5
χ^1 = averaged; $^3J_{\alpha\beta}$ = 6.7, 6.7 Hz	1.8	1.8

	$J_{N\alpha}$ = 4 Hz		$J_{N\alpha}$ = 7 Hz		$J_{N\alpha}$ = 10 Hz	
	R_1	R_2	R_1	R_2	R_1	R_2
One β-proton, 512 t_1 values, $LW(F_1)$ = 5.5 Hz, $LW(F_2)$ = 7.5 Hz						
χ^1 = +60°,180°(T,I); χ^1 = +60°,−60°(V); $^3J_{\alpha\beta}$ = 12.9 Hz	4.4	1.4	3.3	1.3	2.5	1.4
χ^1 = +60°,180° +/−30°;χ^1 = +60°,−60° +/−30°; $^3J_{\alpha\beta}$ = 11.5 Hz	4.3	1.4	3.1	1.4	2.3	1.4
χ^1 = −60° (T,I); χ^1 = 180°(V); $^3J_{\alpha\beta}$ = 3.4 Hz	1.8	1.6	1.4	1.5	1.2	1.5
χ^1 = −60°+/−30°(T,I);χ^1 = 180°+/−30°(V); $^3J_{\alpha\beta}$ = 5.0 Hz	2.0	1.7	1.6	1.6	1.2	1.6
χ^1 = averaged; $^3J_{\alpha\beta}$ = 6.7 Hz	2.5	2.1	1.9	1.9	1.4	1.8
χ^1 = averaged; $^3J_{\alpha\beta}$ = 7.7 Hz	2.9	2.1	2.2	1.9	1.5	1.9

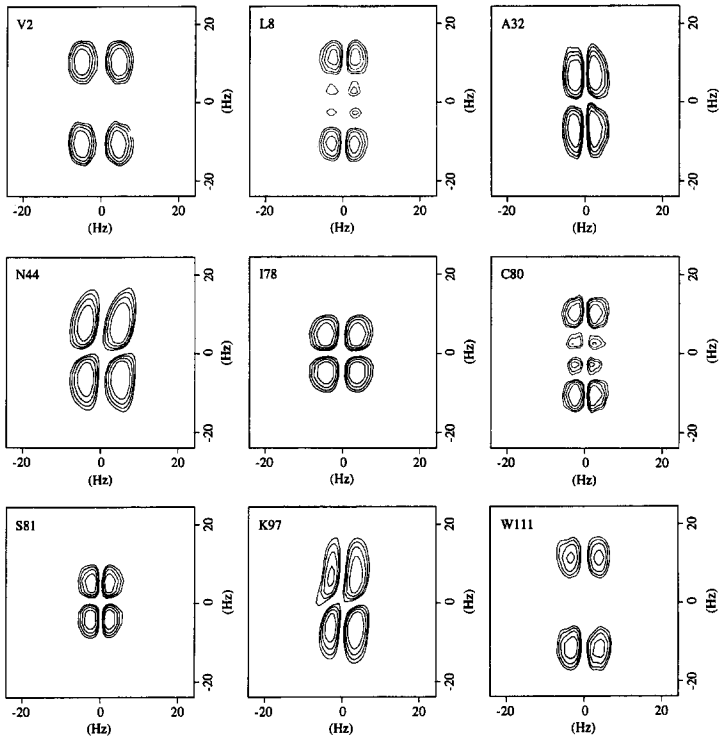

Fig. 4. Examples of NH–αCH cross peaks from the phase-sensitive COSY spectrum of hen lysozyme. The R_1 and R_2 values for these residues are listed in Table 4. On the basis of the peak-shape analysis, V2, L8, C80 and W111 are classified as rectangular, I78 and S81 as square, and A32, N44 and K97 as intermediate.

1991). The cross-peak ratios R_1 and R_2 have been measured for 103 of the 117 non-glycine residues of hen lysozyme and are summarised in Tables 3 and 4. Examples of these cross peaks are shown in Fig. 4. The cross peaks have been classified as square, rectangular, or intermediate according to the rules summarised in Table 2 using the values of R_1, R_2, and $^3J_{NH-\alpha CH}$. The χ^1 values derived from the peak-shape analysis are also summarised in Tables 3 and 4. For example, V2 has an R_1 value of 2.4 and an R_2 value of 1.6. Using the known NH–αCH coupling constant of 10.0 Hz this peak can be classified as rectangular indicating that V2 adopts a χ^1 value of about 180° in solution. N44, on the other hand, has an intermediate peak shape with R_1, R_2 and

TABLE 2
RULES FOR CLASSIFYING CROSS PEAKS AS RECTANGULAR, SQUARE OR INTERMEDIATE ON THE BASIS OF R_1, R_2 AND $^3J_{NH-\alpha CH}$

	$^3J_{N\alpha}$ = 4 Hz		$^3J_{N\alpha}$ = 7 Hz		$^3J_{N\alpha}$ = 10 Hz	
	R_1	R_2	R_1	R_2	R_1	R_2
Rectangular	≥ 3.5	≤ 1.8	≥ 2.5	≤ 1.8	≥ 2.0	≤ 1.8
Square	≤ 2.3	≤ 1.8	≤ 1.8	≤ 1.8	≤ 1.5	≤ 1.8
Intermediate	< 3.5	≥ 1.9	< 2.5	≥ 1.9	< 2.0	≥ 1.9

TABLE 3
COMPARISON OF SIDE-CHAIN CONFORMATION DATA OBTAINED BY PEAK-SHAPE ANALYSIS AND E.COSY METHODS

		E.COSY methods[a]		Peak-shape analysis			
Residue	$J_{N\alpha}$ (Hz)[a]	$J_{\alpha\beta\,(Hz)}$[a]	χ^1 (°)[a]	Type[b]	R_1	R_2	χ^1 (°)[c]
V2	10.0	10.8	180	r	2.4	1.6	180
F3	7.4	3.0/10.0	−60	r	2.7	1.5	−60/180
C6	5.8	3.5/11.5	−60	r	3.5	1.3	−60/180
E7	4.5	6.7/6.4	aver.	a	2.9	2.3	aver.
K13	4.2	9.2/5.1	aver.	r	4.5	1.6	−60/180
H15	9.2	2.6/11.2	−60	r	2.5	1.5	−60/180
D18	5.7	11.0/4.2	180	r	3.5	2.1	−60/180
N19	7.0	6.4/7.3	aver.	a	1.8	1.9	aver.
Y20	5.5	2.3/11.7	180	r	3.2	1.7	−60/180
Y23	8.6	10.9/2.7	−60	r	2.4	1.1	−60/180
N27	5.4	10.3/2.4	−60	r	3.6	1.5	−60/180
W28	6.0	10.7/4.1	−60/180	r	3.4	1.3	−60/180
V29	5.9	11.1	180	r	2.8	1.4	180
C30	3.8	10.8/5.3	180	r	4.5	1.6	−60/180
F34	7.6	5.0/10.7	−60	r	2.8	1.5	−60/180
N37	7.5	8.1/4.2	aver.	a	1.7	1.9	aver.
N39	8.8	10.8/4.5	180	r	2.7	1.7	−60/180
T40	5.4	4.5	60/180	s	1.4	1.4	60/180
T43	9.3	3.7	60/180	s	1.2	1.4	60/180
R45	7.7	6.9/6.7	aver.	a	2.0	2.1	aver.
N46	8.8	4.7/11.2	−60	r	2.3	1.9	−60/180
T47	4.4	2.6	60/180	s	1.6	1.4	60/180
D48	7.7	2.6/3.7	60	s	1.5	1.5	60
T51	9.8	9.3	−60	r	1.9	1.0	−60
D52	9.6	11.6/3.6	−60	r	2.5	1.7	−60/180
Y53	9.6	10.4/3.0	−60	r	2.3	1.5	−60/180
R61	7.2	10.8/5.7	180	r	2.8	1.7	−60/180
C64	8.8	2.7/4.6	60	s	1.2	1.0	60
N65	9.4	11.4/4.5	−60/180	r	2.4	1.5	−60/180
D66	10.0	5.0/4.5	60	s	1.2	1.2	60
R68	9.7	4.8/6.5	aver.	a	1.5	1.9	aver.
T69	9.3	9.3	−60	r	2.0	1.8	−60
S72		5.4/7.6	aver.	a	2.6	3.1	aver.
N74		10.5/3.9	−60/180	r	2.4	1.3	−60/180
L75		12.4/2.1	−60	r	2.6	1.5	−60/180
N77	7.4	8.3/5.9	aver.	a	1.9	2.2	aver.
S85	5.8	5.7/7.4	aver.	a	2.0	1.6	aver.
S86	5.8	6.4/4.1	aver.	a	1.9	1.9	aver.
D87	8.9	11.5/5.1	180	r	2.0	1.2	−60/180
I88	6.5	4.5	60/180	s	1.4	1.3	60/180
T89		9.5	−60	r	6.2	3.0	−60
V92	5.6	10.1	180	r	2.7	1.5	180
N93	4.4	10.8/3.5	−60/180	r	3.3	1.8	−60/180
C94	6.3	4.0/12.2	180	r	3.2	1.4	−60/180

TABLE 3 (continued)

		E.COSY methods[a]		Peak-shape analysis			
Residue	$J_{N\alpha}$ (Hz)[a]	$J_{\alpha\beta\,(Hz)}$[a]	χ^1 (°)[a]	Type[b]	R_1	R_2	χ^1 (°)[c]
V99	5.2	6.3	aver.	a	2.0	1.9	aver.
S100		7.7/4.0	aver.	a	1.6	1.6	aver.
N101	7.0	6.6/5.6	aver.	a	1.7	1.9	aver.
N106		10.5/3.6	−60/180	r	3.5	1.6	−60/180
V109	4.0	8.0	aver.	a	2.6	2.4	aver.
T118	9.8	4.2	60/180	s	1.1	1.5	60/180
D119	6.7	11.7/4.9	180	r	3.5	1.4	−60/180
W123	5.4	2.9/10.6	−60	r	3.7	1.4	−60/180
I124	10.6	4.6	60/180	s	1.0	1.6	60/180
R125	4.4	7.9/6.1	aver.	a	2.5	3.0	aver.
C127	7.7	4.8/11.6	−60	r	2.8	1.6	−60/180
R128	8.0	7.2/7.9	aver.	a	2.0	2.2	aver.

[a] Smith et al., 1991.
[b] r = rectangular cross peak; s = square cross peak; a = intermediate cross peak.
[c] χ^1 classification based on peak-shape analysis.

TABLE 4
COMPARISON OF ADDITIONAL SIDE-CHAIN CONFORMATION DATA OBTAINED BY PEAK-SHAPE ANALYSIS WITH χ^1 VALUES IN THE CRYSTAL STRUCTURES OF HEN LYSOZYME

		Peak-shape analysis				Crystal	
Residue	$J_{N\alpha}$ (Hz)[a]	Type[b]	R_1	R_2	χ^1 (°)[c]	χ^1 (°)[d]	χ^1 (°)[e]
R5		a	2.6	3.0	aver.	−176.4	170.9
L8	5.5	r	3.6	1.6	−60/180	178.3	−179.3
A9	3.7	a	2.5	2.2	alanine		
A10	3.9	a	2.5	2.3	alanine		
A11	4.8	a	2.4	2.2	alanine		
M12	4.6	r	3.6	1.4	−60/180	−82.6	−75.9
R14	4.4	a	2.8	2.4	aver.	−175.1	−77.6
L17	7.6	r	2.5	1.7	−60/180	−77.1	−73.3
R21	6.8	a	2.4	2.1	aver.	−80.1	−64.0
S24		s	1.4	1.9	60	76.2	62.1
A31	3.8	a	2.8	2.7	alanine		
A32	4.8	a	2.3	2.3	alanine		
K33	3.6	r	5.1	2.1	−60/180	171.4	166.8
E35	7.2	r	2.7	1.3	−60/180	−76.0	−72.7
S36	9.6	s	1.3	1.4	60	67.0	66.0
Q41	9.2	r	2.1	1.5	−60/180	−68.4	−68.0
A42	4.5	a	2.5	2.1	alanine		
N44	9.4	a	1.5	2.0	aver.	−73.0	176.3
S50	7.8	s	1.2	1.5	60	69.5	70.2
L56	9.7	r	2.0	1.5	−60/180	−73.3	−76.2

TABLE 4 (continued)

Residue	$J_{N\alpha}$ (Hz)[a]	Peak-shape analysis				Crystal	
		Type[b]	R_1	R_2	χ^1 (°)[c]	χ^1 (°)[d]	χ^1 (°)[e]
Q57	6.3	r	3.2	1.5	−60/180	−67.9	−64.6
I58	8.0	r	2.5	1.4	−60	−70.5	−73.4
S60	5.1	s	1.5	1.4	60	72.4	59.0
I78	8.0	s	1.1	1.2	60/180	−159.9	50.1
C80	3.6	r	3.7	1.4	−60/180	−62.4	−61.4
S81	3.6	s	1.8	1.5	60	59.6	59.8
A82	5.4	a	2.4	2.0	alanine		
L83	7.2	r	2.6	1.2	−60/180	−57.0	−58.7
L84	9.2	r	2.5	1.6	−60/180	−58.3	−64.2
A90	4.2	a	2.8	2.5	alanine		
K96	4.4	a	2.0	2.2	−60/180	−73.5	−67.0
K97	6.5	a	2.2	2.5	aver.	−97.2	173.6
I98		r	2.6	1.5	−60	−60.3	−70.2
N103	8.2	a	1.7	2.0	aver.	−51.1	−61.7
M105	7.4	r	2.5	1.5	−60/180	−75.6	−65.6
A107	4.2	a	2.8	2.4	alanine		
W108	9.6	r	2.4	1.5	−60/180	−78.6	−71.1
W111	7.1	r	3.1	1.8	−60/180	178.1	179.3
R112	4.5	a	2.2	2.5	aver.	−171.8	−174.0
N113	5.8	a	1.7	1.9	aver.	−65.1	−73.1
R114	9.6	r	2.4	1.2	−60/180	−44.9	−53.0
C115	9.8	r	2.3	1.4	−60/180	−60.9	−58.5
K116		r	3.3	1.9	−60/180	−166.7	−179.1
V120	4.6	s	1.6	1.5	180	−61.9	−66.6
Q121	5.0	a	2.4	2.9	aver.	−99.3	−72.7
A122	3.7	a	2.8	2.8	alanine		
L129	9.0	r	2.4	1.8	−60/180	−46.6	−74.4

[a] Smith et al., 1991.

[b] r = rectangular cross peak; s = square cross peak; a = intermediate cross peak.

[c] χ^1 classification based on peak-shape analysis.

[d] 2.0-Å tetragonal structure (Handoll, 1985).

[e] 1.4-Å triclinic structure (Mason, personal communication).

$^3J_{NH-\alpha CH}$ values of 1.5, 2.0 and 9.4, respectively, indicating that the side chain is undergoing motional averaging about χ^1. All the alanine cross peaks give the expected intermediate peak shape. Using this approach χ^1 information has been obtained for 93 of the possible 102 residues of hen lysozyme. Information for the nine remaining residues was not obtained either because of severe cross-peak overlap or because of very low cross-peak intensity.

αCH–βCH coupling constants, measured from E.COSY experiments, have been reported for 57 residues in hen lysozyme (Smith et al., 1991). The side chains of 41 of these residues are predicted to be in one of the preferred staggered rotamers whereas the side chains of the remaining 16 residues are predicted to be undergoing motional averaging about χ^1 (Smith et al., 1991). The NH–αCH cross-peak fine structure could be analysed for 56 of these 57 residues and this

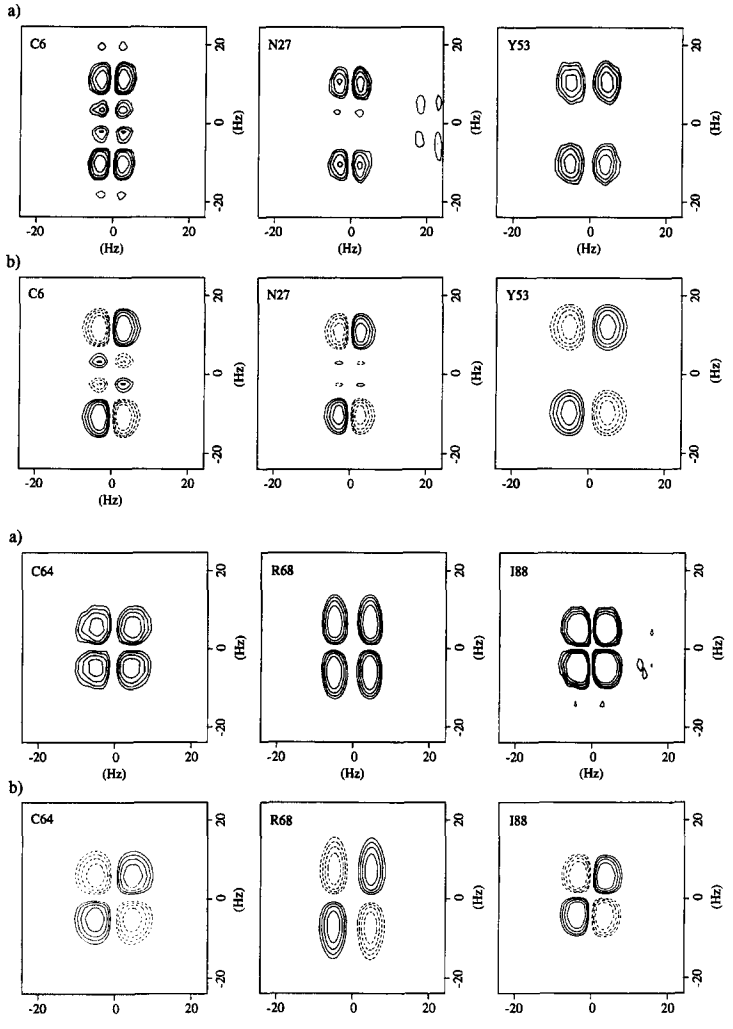

Fig. 5. Comparison of experimental (a) and simulated (b) NH–αCH cross peaks for six residues of hen lysozyme. The NH–αCH and αCH–βCH coupling constants used in the simulations are listed in Table 3. The NH linewidths used in the F_2 dimension were obtained from the NH–αCH coupling-constant fitting procedure (Smith et al., 1991). A linewidth of 5.5 Hz was used in the F_1 dimension.

analysis has predicted the same side-chain conformation for all but one of these residues. NH–αCH cross peaks simulated using the values of $^3J_{NH-\alpha CH}$ and $^3J_{\alpha CH-\beta CH}$ previously reported, are compared with the experimental cross peaks in Fig. 5; the agreement is striking. K13 is the only residue for which a different χ^1 classification is obtained by the two methods. The NH–αCH cross-peak fine structure of K13 is distinctively rectangular ($R_1 = 4.5$ and $R_2 = 1.6$) but the difference in the two αCH–βCH coupling constants was found to be less than 5 Hz (9.2 Hz and 5.1 Hz) in the previous study (Smith et al., 1991). The NH–αCH cross peak of K13 was simulated using various values of $^3J_{\alpha CH-\beta CH}$ as shown in Fig. 6. The cross peak simulated with values of 9.2 and 5.1 Hz is very different in appearance to the experimental peak; this simulated peak gives R_1 and R_2 values of 3.5 and 2.4 which are characteristic of an intermediate peak. Better agreement

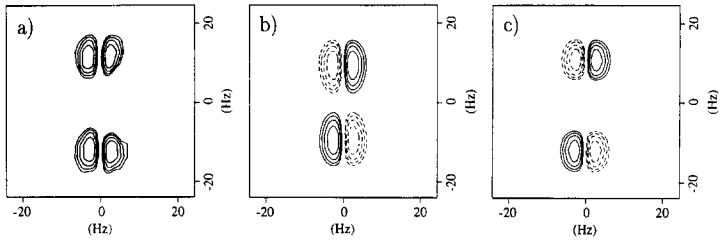

Fig. 6. (a) Experimental NH–αCH cross peak of K13. (b) NH–αCH cross peak simulated with $^3J_{\alpha CH-\beta CH}$ = 5.1 and 9.2 Hz, the values measured from the E.COSY spectrum (Smith et al., 1991). (c) NH–αCH cross peak simulated with $^3J_{\alpha CH-\beta CH}$ = 5.0 and 11.5 Hz.

is obtained with αCH–βCH coupling constants of 11.5 and 5.0 Hz. The close agreement between χ^1 classifications for 56 residues made using E.COSY methods and cross-peak shape analysis indicates that the latter is a reliable method for determining approximate χ^1 values in proteins.

χ^1-angle information for an additional 37 residues has been obtained from the peak-shape analysis procedure. αCH–βCH coupling constants for these residues were not obtained previously from E.COSY methods because of the lack of firm βCH assignments or because of overlap in the E.COSY spectrum. The χ^1-angle assignments for these residues are listed in Table 4. Of the 37 residues, 20 give rise to rectangular peaks, 7 to square peaks and 10 to intermediate peaks. In the previous study of side-chain conformation in hen lysozyme it was found that residues which adopt a single χ^1 value in solution are also found to have this value of χ^1 in the X-ray structure (Smith et al., 1991). The χ^1 values obtained from the 2.0-Å tetragonal and 1.4-Å triclinic structures of hen lysozyme are listed for the 37 residues in Table 4. All residues classified as square or rectangular on the basis of cross-peak analysis are found to have χ^1 values in both X-ray structures which are entirely consistent with the χ^1 classification from this analysis. In the earlier study of side-chain conformation it was also found that residues undergoing motional averaging about χ^1 in solution almost always have side chains which are on the surface of the protein with a high solvent accessibility (Smith et al., 1991). The 10 additional residues found in this study to have disordered side chains in solution are all located on the protein surface. It is interesting to note that three of the 10 residues are found to have different χ^1 values in the tetragonal and triclinic structures; a similar situation was observed for half of the residues undergoing motional averaging about χ^1 in the earlier study of hen lysozyme (Smith et al., 1991).

The peak-shape analysis method can also provide information that is useful at an early stage of the assignment task. For example, an NH–αCH cross peak belonging to an alanine must have an intermediate peak shape. A residue with two β-protons, giving rise to a square NH–αCH cross peak, would be expected to have a pair of very weak peaks in the αCH–βCH region of the COSY spectrum. A valine that shows a rectangular NH–αCH peak is more likely to give rise to a pair of strong αCH–γCH_3 peaks in RELAY or HOHAHA spectra than a valine with a square NH–αCH peak.

The peak-shape analysis method for the qualitative assessment of χ^1 values should be applicable to proteins of up to at least 130 residues. Indeed, the characteristic peak shapes described here can be seen in published COSY spectra of acyl carrier protein and plastocyanin (Holak and Prestegard, 1986; Driscoll et al., 1987). If the protein of interest has linewidths that are significantly different from those used here for lysozyme or if the COSY spectrum was collected or processed

with very different digital resolution or window functions, then cross peaks for the different χ^1 categories should be simulated using an appropriate set of parameters before the analysis is carried out.

CONCLUSION

A method, based on the analysis of NH–αCH cross-peak shapes, for obtaining qualitative information about χ^1 values has been presented. Cross peaks with a square or rectangular shape arise when a residue has a well-defined conformation about χ^1. Ambiguities in the χ^1 classification (i.e. 180° versus −60°) can only be resolved on the basis of additional NOE information (Wagner et al., 1987; Arseniev et al., 1988). Cross peaks with an intermediate shape are characteristic of motional averaging about χ^1. This type of qualitative information about χ^1 values is of use in the early stages of spectral assignment, in the stereospecific assignment of β-protons and in structure calculations. The method has been proven reliable using a group of 56 residues from hen lysozyme for which χ^1 information had been derived from earlier E.COSY measurements (Smith et al., 1991). In addition, χ^1 information has been obtained for another 37 residues of hen lysozyme.

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