Volume 255, number 2, 369-371 FEB 07632 September 1989

Improved resolution in ^{1}H-detected ^{1}H-^{15}N correlation experiments*

T.J. Norwood, J. Boyd and I.D. Campbell

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, England

Received 21 July 1989

The determination of protein structure by NMR is restricted at molecular masses above 10 kDa by overlapping resonances. One way of overcoming this problem is to label the protein with ^{15}N. The conventional way to record ^{15}N spectra is to use heteronuclear multiple-quantum coherence. We present here an alternative approach based on ^{15}N single-quantum coherence. This is shown to have substantial advantages over the multiple-quantum method, including better F_1 resolution.

Nuclear magnetic resonance; Protein structure; Labeling, ^{15}N

The use of NMR for the elucidation of protein structure has become established in the last few years. Typically this is achieved by using a combination of the two-dimensional ^{1}H NMR techniques COSY and NOESY [1-5]. With increasing molecular weight the resonance assignment problem becomes more difficult because of spectral overlap and larger linewidths. An elegant solution to this problem is to use isotopically labelled proteins. ^{15}N has proved to be particularly useful in this respect since it has a much larger chemical shift dispersion than protons. ^{15}N chemical shift is conventionally encoded in the second dimension of a two-dimensional experiment in which protons are both excited and detected to maximise sensitivity. These experiments use either heteronuclear ^{1}H-^{15}N multiple-quantum coherence [6-17] (HMQC), or ^{15}N single-quantum coherence (SQC) [18] to encode ^{15}N chemical shifts. All published studies of ^{15}N-labelled proteins have been undertaken utilising the former technique. In this communication we show that experiments based on the ^{15}N SQC technique have several advantages, including improved F_1 resolution and longer transverse relaxation times, than those based on HMQC [19].

Correspondence address: T.J. Norwood, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, England

* A contribution from the Oxford Centre for Molecular Sciences.

The basic HMQC and ^{15}N SQC experiments are given in fig.1. The improvement in resolution obtained by using ^{15}N SQC results from a reduction in the effective linewidths of peaks in the F_1 (^{15}N) dimension of the spectrum. This arises from two sources: removal of homonuclear ^{1}H scalar couplings and reduction in the transverse relaxation rate of the coherences evolving during the t_1 evolution period.

The evolution periods of experiments utilising either ^{15}N SQC or ^{1}H-^{15}N HMQC to encode ^{15}N chemical shift typically incorporate a 180°(^{1}H) pulse at $t_{1/2}$. Although this refocusses all of the scalar couplings experienced by ^{15}N SQC in a protein, it does not remove those experienced by the HMQC, which will exhibit the homonuclear multiplet of its active proton. Thus a peak in an HMQC spectrum will exhibit a multiplet in the F_1 dimension while the corresponding peak in a ^{15}N SQC spectrum will not.

^{15}N SQC relaxation rates, and hence linewidths, are usually less than their HMQC counterparts. This can be explained if one assumes that relaxa-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

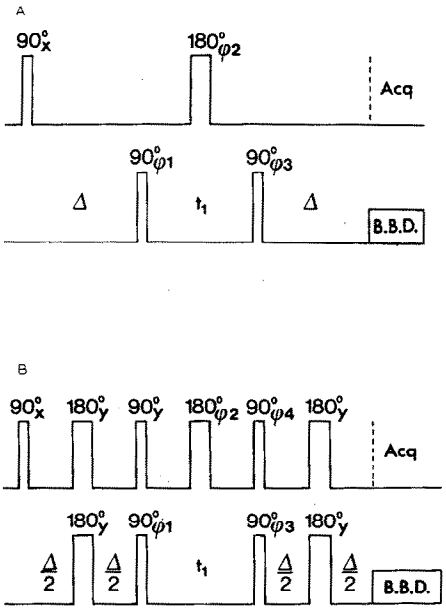

Fig.1. Pulse sequences for heteronuclear ^{1}H–^{15}N spectroscopy based upon (A) ^{1}H observed heteronuclear multiple-quantum coherence, and (B) ^{15}N single-quantum coherence. Phase cycling used for (A): $\phi1 = (x,-x)$; $\phi2 = 2(x,y,-x,-y)$; $\phi3 = 8(x-x)$; receiver = $(x,-x) + 2(x,-x) + 8(x,-x)$; for (B) $\phi1 = (x,-x)$; $\phi2 = 2(x,y,-x,-y)$; $\phi3 = 8(x,-x)$; $\phi4 = 8(x,-x)$; receiver = $(x,-x) + 2(x,-x)$. We have found that more extensive phase cycling is required for natural abundance ^{15}N studies. In both cases $\Delta = 0.5$ J, where J is the one-bond ^{1}H–^{15}N coupling constant.

tion is predominantly dipolar in origin. The efficiency of dipolar relaxation is proportional to γ^2, and consequently ^{15}N will relax less efficiently than ^{1}H since $\gamma_N = 0.1\ \gamma_H$. A ^{15}N SQC arising from a backbone amide of a protein will relax predominantly with its bonded proton ($r = 1.0$ Å) since all other nuclei with which it might relax are substantially further away ($r > 2.2$ Å). A ^{15}N–^{1}H HMQC is created between an amide ^{15}N and its bonded ^{1}H. In the slow tumbling limit their mutual relaxation vanishes, effectively removing the ^{15}N contribution to the relaxation of the coherence. As a result the coherence will relax primarily due to the homonuclear dipolar relaxation of the active proton. Due to the difference of gyromagnetic ratios noted above ^{15}N SQC will usually relax less efficiently than HMQC and will consequently have narrower lines. By using these arguments it can be shown that the ratio of HMQC to ^{15}N SQC linewidths for an α-helix is expected to be within the range 1:1 to 3:1. In the current study we have

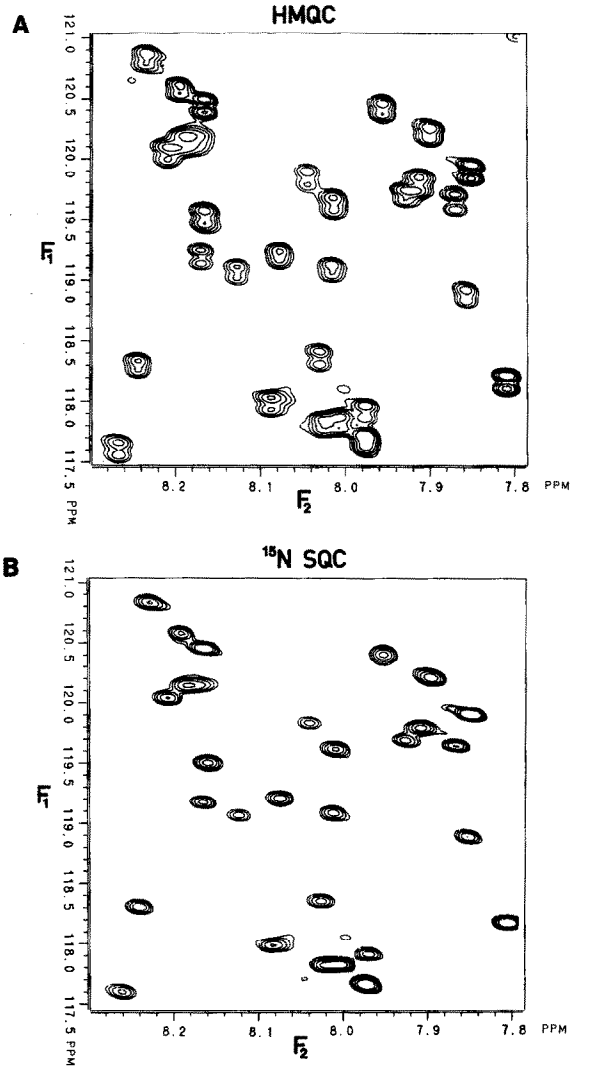

Fig.2. Corresponding regions of (A) HMQC COSY and (B) ^{15}N SQC COSY ^{1}H–^{15}N spectra of a 5 mM solution of the c-subunit of the F_1–F_0 ATP-synthase of *E. coli* [22] in trifluoroethanol-d_2. All peaks shown correspond to one-bond ^{1}H–^{15}N correlations. The ^{15}N SQC spectrum clearly has better F_1 resolution than its HMQC counterpart. Both spectra are plotted to the same vertical scale. All experiments were carried out on a home-built spectrometer operated by a GE 1280 computer and using an 11.8 T vertical bore Oxford Instruments magnet operating at 500.1 MHz for ^{1}H and 50.7 MHz for ^{15}N. Phase-sensitive data were acquired by incrementing the phase of the second 90°(^{15}N) pulse in each case [23]. In each case $F_2 = 5208$ Hz and $F_1 = 1666$ Hz, and 512 t_1 increments with 128 transients each were acquired. $\Delta = 5.25$ ms, $\tau = 30$ ms.

observed this ratio to be between 1:1 and 2:1.

The exact decrease in the effective F_1 linewidth achieved by using ^{15}N SQC instead of HMQC will

vary from peak to peak since it depends on both the size of the couplings removed and the reduction in transverse relaxation rate achieved. The reduction in effective linewidth leads, of course, to a corresponding increase in signal-to-noise ratio.

The major disadvantage of the ^{15}N SQC technique is that it requires a greater number of pulses. This may result in loss of signal intensity (typically 20%) due to pulse imperfections, although in most cases there is still an overall gain in intensity. It may also make solvent suppression more difficult.

A comparison of HMQC and ^{15}N SQC spectra is given in fig.2. The F_1 resolution of the SQC spectrum is clearly substantially better than its HMQC counterpart.

Both the basic HMQC and ^{15}N SQC experiments can be adapted to incorporate homonuclear ^1H COSY, HOHAHA and NOESY in order to exploit fully the ^{15}N chemical shift. In both HMQC and ^{15}N SQC, HOHAHA [20,21] and NOESY [4] can be incorporated by the addition of the appropriate 'module' to the end of the basic pulse sequence [14]. In the case of COSY only a 90°(^1H) pulse need be added to the end of the basic HMQC experiment [11] but the ^{15}N SQC experiment requires the replacement of the second Δ period of the basic experiment with the sequence: (τ/2-180°(^1H)-(τ/2)-90°(^1H); broadband ^{15}N decoupling is turned on after a time Δ. The delay τ is set to 0.25 J for homonuclear ^1H scalar couplings. The addition of this delay to the ^{15}N SCQ COSY experiment makes it longer than its HMQC counterpart, and as a consequence it will suffer greater signal attenuation due to transverse relaxation. However, this loss of signal intensity is compensated for by the *increase* in signal intensity resulting from the decrease of the effective F_1 linewidth, as can be seen from fig.2. For all other experiments discussed, those based upon ^{15}N SQC will produce spectra that exhibit better signal-to-noise ratios than those based on HMQC when experimental conditions are the same.

We have shown that in many cases SQC experiments have advantages over their HMQC counterparts for heteronuclear ^1H-^{15}N spectroscopy of proteins. In particular, the F_1 resolution of a spectrum produced by the SQC technique is often considerably better than the corresponding HMQC spectrum. Most ^{15}N SQC experiments also have better signal-to-noise ratios than their HMQC counterparts. It should thus be possible both to increase the size of proteins that can usefully be studied by NMR, and to reduce the concentration of those that can be studied by heteronuclear ^1H-^{15}N techniques.

Acknowledgements: We wish to acknowledge Nick Soffe's continual support, and Jane Heritage for preparing the protein. We thank SERC for financial support (T.J.N.).

REFERENCES

[1] Jeener, J. (1971) Ampere International Summer School, Basko, Polje, Yugoslavia.
[2] Aue, W.P., Bartholdi, E. and Ernst, R.R. (1976) J. Chem. Phys. 64, 2229-2246.
[3] Ernst, R.R., Bodenhausen, G. and Wokaun, A. (1987) Principles of Nuclear Magnetic Resonance in One and Two Dimensions, Clarendon, Oxford.
[4] Jeener, J., Meier, B.H., Bachmann, P. and Ernst, R.R. (1979) J. Chem. Phys. 71, 4546-4553.
[5] Wüthrich, K. (1986) NMR of Proteins and Nucleic Acids, Wiley-Interscience, New York.
[6] Muller, L. (1979) J. Am. Chem. Soc. 101, 4481-4484.
[7] Redfield, A.G. (1983) Chem. Phys. Lett. 96, 537-540.
[8] Bax, A., Griffey, R.H. and Hawkins, B.L. (1983) J. Magn. Reson. 55, 301-315.
[9] Sklener, V. and Bax, A. (1987) J. Magn. Reson. 74, 469-479.
[10] Glushhka, J. and Cowburn, D. (1987) J. Am. Chem. Soc. 109, 7879-7881.
[11] Clore, G.M., Bax, A., Wingfield, P. and Gronenborn, A.M. (1988) FEBS Lett. 238, 17-21.
[12] Bax, A. and Summers, M.F. (1986) J. Am. Chem. Soc. 108, 2093-2094.
[13] Bax, A. and Marion, D. (1988) J. Magn. Reson. 78, 186-191.
[14] Gronenborn, A.M., Bax, A., Wingfield, P.T. and Clore, G.M. (1989) FEBS Lett. 243, 93-98.
[15] Griffey, R.H. and Redfield, A.G. (1985) Biochem. 24, 817-822.
[16] Stockman, B.J., Reily, M.H., Westler, W.M., Ulrich, E.L. and Markley, J. (1989) Biochem. 28, 230-236.
[17] Bax, A., Kay, L.E., Sparks, S.W. and Torchia, D.A. (1989) J. Am. Chem. Soc. 111, 408-409.
[18] Bodenhausen, G. and Ruben, D.J. (1980) Chem Phys. Lett. 69, 185-189.
[19] Norwood, T.J., Boyd, J. and Campbell, I.D. (1989) 30th Experimental NMR Conference (ENC), Asilomar, CA, USA.
[20] Braunschweiler, L.R. and Ernst, R.R. (1983) J. Magn. Reson. 53, 521-528.
[21] Davies, D.G. and Bax, A. (1985) J. Am. Chem. Soc. 107, 2821-2822.
[22] Moody, M., Jones, P.J., Carver, J.A., Boyd, J. and Campbell, I.D. (1987) J. Mol. Biol. 193, 759-774.
[23] States, D.J., Haberkorn, R.A. and Ruben, D.J. (1982) J. Magn. Reson. 48, 286-292.