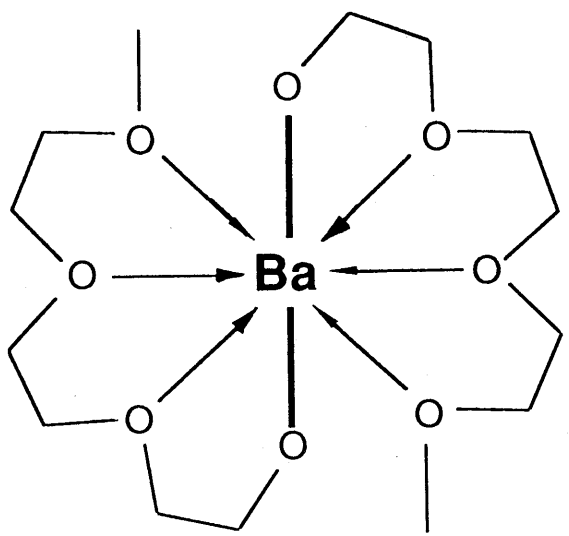

FIGURE 5 Proposed structure of $Ba[O(CH_2CH_2O)_3Me]_2$.

Acknowledgements

We gratefully acknowledge DARPA contract number MDA 972-88-J-1006 for the generous support of this work, Dr. Alexander Sytnik for assistance with the florescence experiments, and Mr. Dick Roche for the illustrations.

References

1. Aristotle, *Physica*, 384 B.C.
2. I. Newton, *Optical Lectures*, 1728.
3. W. Ostwald, *Color Theory and Color Standardization*, 1931.
4. J. W. von Goethe, *Beträge zur Optik*, 1791.
5. K. Nassau, *The Physics and Chemistry of Color*, 1983.
6. Polonius to Ophelia (*Hamlet*, Act I, Scene 3), "These blazes, giving more light than heat."
7. D. H. Kabakjian, *Physical Review*, **57**, 1940, p. 700.
8. W. S. Rees, Jr. and D. A. Moreno, *J. Chem. Soc., Chem. Commun.*, **1991**, 1759 - 60.

Modification of a Bruker AM-600 Spectrometer for Double and Triple Resonance Three- and Four-Dimensional Experiments Illustrated with Chicken Adenylate Kinase Resonance Assignments.

Ed S. Mooberry, Arthur S. Edison, Frits Abildgaard, John L. Markley
Department of Biochemistry
University of Wisconsin, 420 Henry Mall
Madison, WI 53706

and

In-Ja L. Byeon, Ming-Daw Tsai
Department of Chemistry
Ohio State University, 140 W. 18th Avenue
Columbus, OH 43210.

ABSTRACT

A Bruker AM-600 spectrometer with an intermediate frequency of 451 MHz has been modified for double and triple resonance studies of 100% ^{13}C and ^{15}N enriched proteins. In order to obtain three and four channel operation, a Bruker BSV-3 X-nucleus decoupler was modified and additional hardware was constructed for phase shifting the third and fourth channels. Results are shown for chicken adenylate kinase (AK_1) complexed to a substrate analog - P^1,P^5-bis-adenosine pentaphosphate (AP_5A). Adenylate kinase is a small enzyme (21.7 kDa for AK_1) which catalyses the reaction of MgATP and AMP to give MgADP and ADP. A number of questions remain regarding the catalytic mechanism even after extensive studies by a variety of techniques. The ATP binding site of AK is not completely located and very little is known of the reaction transition state. AK contains a large portion of α-helical structure making resonance assignments difficult; increased resolution was obtained from 3D HNCO, HNCA, HN(CO)CA, CT(constant time)-HCACO, CT-HCA(CO)N, H(CA)CON, HCCH-COSY, HCCH-TOCSY, ^1H-^{15}N NOESY, and 4D CT-HCACON experiments.

Introduction

Three- and four-dimensional (3D and 4D) nuclear magnetic resonance (NMR) techniques have made possible the complete assignment of all NMR active spin 1/2 nuclei in some small proteins (to 20 to 25 kD).[1] Adenylate kinase is a small enzyme (21.7 kDa for AK_1) which catalyses the reaction of MgATP and AMP to give MgADP and ADP. A number of questions remain regarding the catalytic mechanism even after extensive studies by x-ray crystallography, site selective mutagenesis and NMR spectroscopy. The ATP binding site of AK is not completely located and very little is known of the reaction transition state.[2-4] In order to understand these problems the 3D NMR structure determination of AK_1 complexed with a substrate analog is the eventual goal.

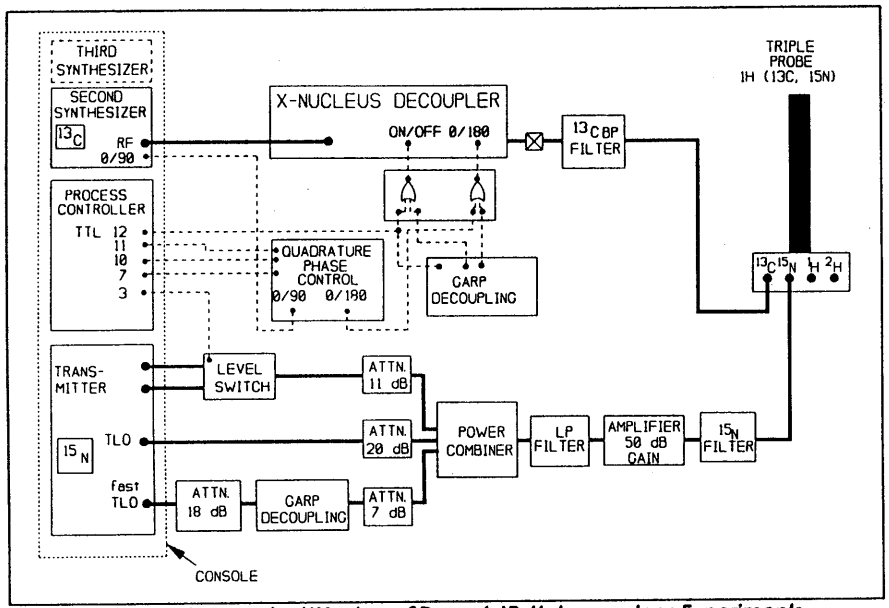

Figure 1: Wiring diagram for HN... type 3D- and 4D-Heteronuclear Experiments.

Experimental:

Recombinant chicken adenylate kinase produced from E. coli was labeled uniformly with ^{15}N and $^{13}C/^{15}N$. NMR samples were prepared in either 100% D_2O or 90% H_2O/10% D_2O in 5mm diameter samples.

Figure 1 shows the wiring diagram for HN... type 3D- and 4D-experiments on a Bruker AM-600 spectrometer. Radio frequency (RF) coaxial cables and digital transistor-transistor logic (TTL) lines are shown as solid and dashed lines, respectively. The ^1H observe and ^2H lock channels were wired as for the usual inverse detection experiment with ^{13}C and ^{15}N bandstop filters in line for the lock channel and a ^1H bandpass filter in the observe channel (pulses provided by the decoupler). Similar circuitry along with a third synthesizer was used for the fourth channel. By interchanging ^{13}C and ^{15}N channels the 3D and 4D experiments requiring 100% D^2O as a solvent may be done.

Incorporated into the scheme was a Bruker BSV-3 X-nucleus decoupler which was modified to include 180° phase shift capability; the 90° phase shift was implemented on the 250 MHz second frequency synthesizer. RF switches were obtained from Doty Scientific; these require more current for switching than normal TTL chips can supply so the 50 ohm lines from the Bruker process controller were used. An AM timer box and GARP decoupling boxes were obtained from Tschudin Associates.[5] The timer box, which allows for double- buffered data acquisition and bypasses software reloading for each

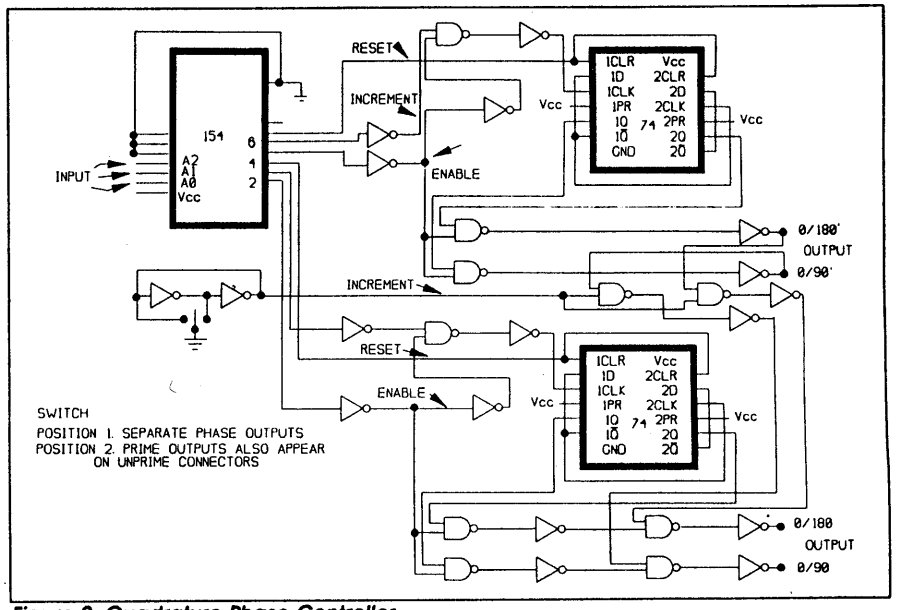

Figure 2: Quadrature Phase Controller

acquisition, results in large time savings. 3D data were collected by a Bruker macro to achieve States-TPPI detection in ω_2.[6]

The Bruker AM console does not provide phase control of the RF signal on the third

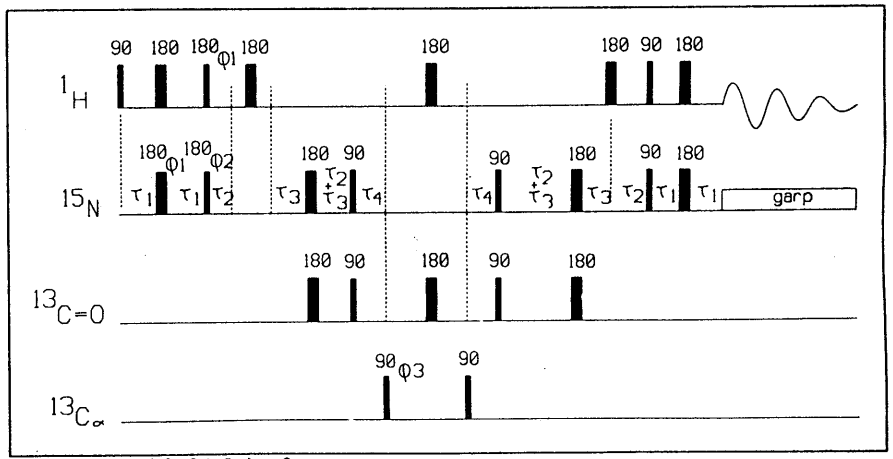

Figure 3: HN(CO)CA Pulse Sequence.

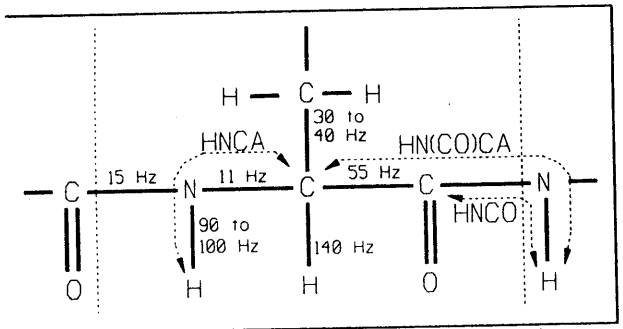

Figure 4: One Bond Heteronuclear Coupling Constants

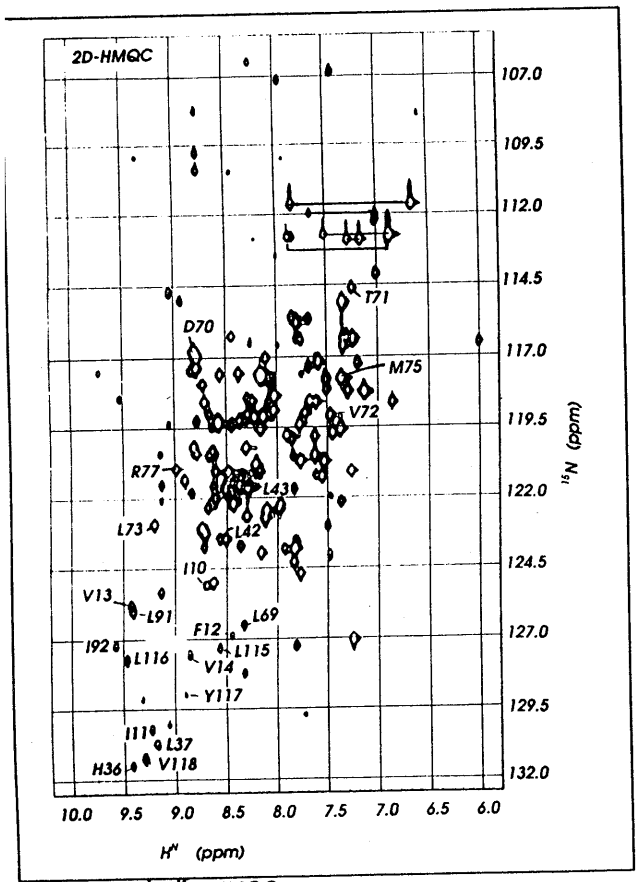

Figure 5: 2D ^{1}H-^{15}N HMQC.

and fourth channels. To achieve this the console was modified as shown in Figure 1. The quadrature phase shifts (0°,90°,180°, and 270°) were produced by the circuitry shown in Figure 2. Three input TTL lines from the Bruker process controller were used to control two sets of phase shifts.

Voltage transients have plagued 3D and 4D data collection aborting several experiments. For this study we collected individual 3D slices as 2D files. However, a United Power motor-generator for power line isolation has been installed so that complete 3D files may be collected as a single serial file without interruption. (A macro is still needed for 4D data collection.)

Figure 3 shows the pulse sequence used for the 3D HN(CO)CA[7] experiment. Similar sequences were used for 3D HNCO,[5] HNCA[5] and other 3D- and 4D-experiments. The RF phase of the first Cα pulse was controlled by the quadrature phase controller shown in Figure 2.

Figure 4 illustrates the

coupling constants of the peptide residues in a protein. The paths of magnetization transfer for HNCA, HNCO, and HN(CO)CA are illustrated as well. The tau values of Figure 3 are various fractions or combinations of these coupling constants.

The commercial NMR software program Felix (version 2.01) on a Silicon Graphics 4D/25 was used to Fourier transform the data obtained. The raw and processed data were stored on a Genesis Systems optical drive cartridge holding 325 MBytes per side.

Results and Discussion

Figure 5 shows the 2D ^{1}H-^{15}N HMQC[8] spectrum for AK_1 complexed to $MgAP_5A$ in 90% H_2O/10% D_2O. Tentative assignments are shown; horizontal lines connect cross peaks from the Gln and/or Asn NH_2 groups. Figure 6 shows a single 3D slice from the 3D ^{1}H-^{15}N NOESY-HMQC[8] experiment giving spatial information about AK_1.

Selected ^{15}N chemical shift slices from the 3D HNCA and HN(CO)CA experiments of AK (uniformly labeled with ^{15}N and ^{13}C) complexed with $MgAP_5A$ are shown in Fig. 7. This figure illustrates the sequential backbone assignment for the Ala-79-Leu-69 peptide stretch of AK. The ^{15}N chemical shift of each slice is given at the top of the figure. Intraresidue cross peaks in the HNCA data are labeled with their amino acid sequences while sequential cross peaks in the HNCA and HN(CO)CA are boxed. The starting point of the assignment was Leu-73 which had been assigned previously from ^{1}H-^{15}N HMQC data on AK labeled with ^{15}N-Leu and ^{13}C-Val. NH-NH connectivities in this region were observed as well indicating α-helix formation.

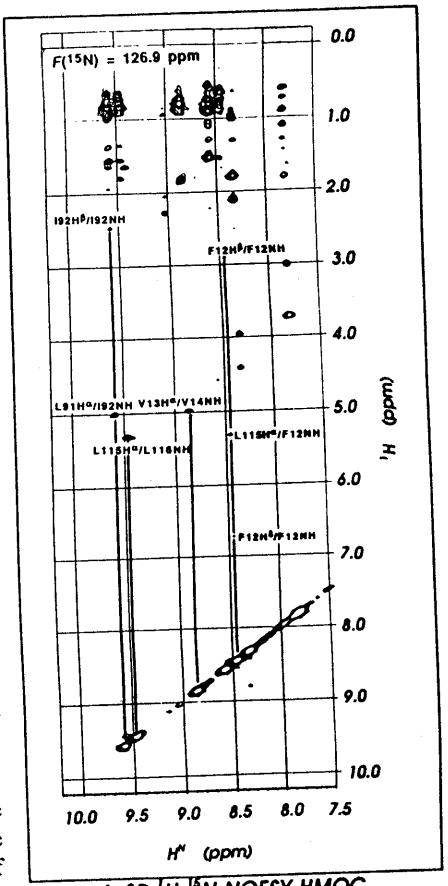

Figure 6: 3D ^{1}H-^{15}N NOESY HMQC

The following 3D- and 4D- NMR experiments are being used to assign the AK_1 resonances in 90%H_2O/10%D_2O: HNCO, HNCA, HN(CO)CA, ^{1}H-^{15}N NOESY-HMQC and in 100% D_2O: HCACO,[5] HCA(CO)N,[5] and 4D HCACON.[9] Future studies will include 4D ^{13}C-^{15}N, ^{13}C-^{13}C, and ^{15}N-^{15}N NOESY data for distance constraints for the structure calculation as well as to verify the assignments.

Acknowledgement:

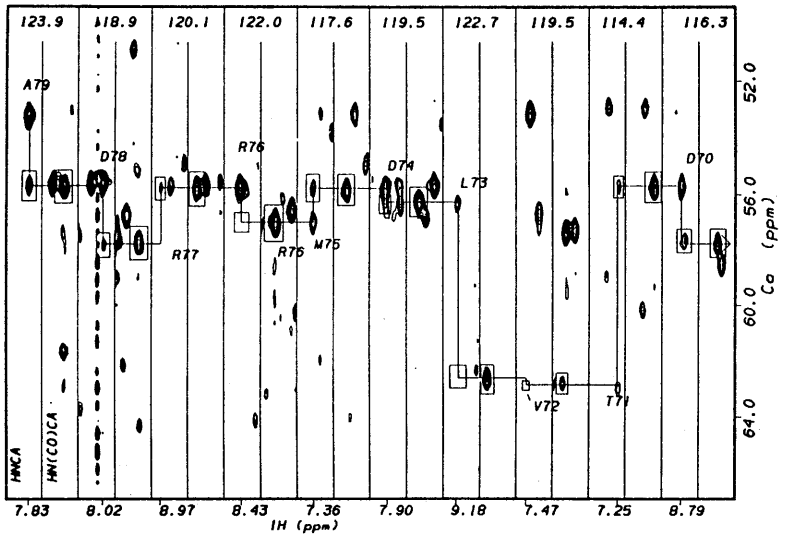

Figure 7: Alternating Slices of the 3D HNCA and HN(CO)CA.

This study made use of the National Magnetic Resonance Facility at Madison which is supported in part by NIH grant RR02301 from the Biomedical Research Technology Program, Division of Research Resources. Equipment in the Facility was purchased with funds from this program, the University of Wisconsin, the NSF Biological Instrumentation Program (grant DBM-8415048) the NIH Shared Instrumentation Program (grant 1R02781), and the U.S. Department of Agriculture.

References:

1. G. M. Clore and A. M. Gronenborn, *Science*, **252** (1991) 1390-1399.
2. H. Yan, T. Dahnke, B. Zhou, A. Nakazawa, and M.-D. Tsai, *Biochemistry*, **29** (1990) 10956-10964.
3. H. Yan, Z. Shi, and M.-D. Tsai, *Biochemistry*, **29** (1990) 6385-6392.
4. M.-D. Tsai and H. Yan, *Biochemistry*, **30** (1991) 6806-6818.
5. L.E. Kay, M. Ikura, R. Tschudin, and A. Bax, *J. Magn. Reson.*, **89** (1991) 496-514.
6. J.-P. Simorre and D. Marion, *J. Magn. Reson.*, **89** (1990) 191-197.
7. M. Ikura, L.E. Kay, and A. Bax, *Biochemistry*, **29** (1991) 4659-4667.
8. D. Marion, L.E. Kay, S. W. Sparks, D. Torchia, and A. Bax, *J. Am. Chem. Soc.*, **111** (1989) 1515-1517.
9. L.E. Kay, M. Ikura, G. Zhu, and A. Bax, *J. Magn. Reson.*, in press.

LIST OF CONFERENCE PARTICIPANTS

Ralph Becker
3702 Purdue St.
Houston TX 77005
USA

Michael J. Bennett
510 W. 19th St.
Merced CA 95340
USA

Dirck Benson
Bristol-Myers Squibb, Box 191
New Brunswick NJ 08903
USA

Dennis Burke
Physics Department ABB241
McMaster University
Hamilton, O. L85 4M1
CANADA

J. W. Cable
P. O. Box 2008
Oak Ridge National Laboratory
Oak Ridge TN 37831-6393
USA

Gregory Choppin
Department of Chemistry
Florida State University
Tallahassee FL 32306
USA

Paul Cloessner
Westinghouse Savannah River Co.
Bldg 773-A
Aiken SC 29802
USA

Paul Cottle
Department of Physics
Florida State University
Tallahassee FL 32306
USA

Jack E. Crow
National High Magnetic Field Lab.
1800 E. Paul Dirac Dr.
Tallahassee FL 32310
USA

John W. Dawson
CHEMLABS, Inc.
1307 Greenview Dr.
Brentwood TN 37027
USA

Dan Decman
Lawrence Livermore National Lab.
Mail Stop L-396
Livermore CA 94550
USA

Raymond Dewberry
Westinghouse Savannah River Co.
773-A Savannah River Laboratory
Aiken SC 29802
USA