

Two Dimensional Double-Quantum and COSY Spectra of Porcine Adenylate Kinase

Paul Rösch and Karl-Heinz Gross

Max-Planck-Institut für Medizinische Forschung, Jahnstr. 29, D-6900 Heidelberg

Z. Naturforsch. **39c**, 738–741 (1984); received April 11, 1984

Adenylate Kinase, COSY, Double-Quantum Spectroscopy, Two-Dimensional Spectroscopy, Protein NMR, Phosphotransferases

¹H double-quantum and COSY NMR-spectra of the porcine protein adenylate kinase have been obtained in order to assign aromatic spin systems within this protein, which contains seven tyrosyl, five phenylalanyl, and two histidyl residues. Double-quantum spectroscopy in its simple form allowed the assignment of five connectivities in the aromatic region and therefore proved inferior to COSY, which allowed the assignment of connectivities within nine aromatic residues.

In the recent years several reports on two-dimensional (2D) spectra of proteins have appeared. These reports have dealt mainly with proteins such as hen egg white (HEW) lysozyme, bovine pancreatic trypsin inhibitor (BPTI), and snake venoms, which already have the attributes of model compounds with respect to nuclear magnetic resonance experiments. Restrictions on the use of 2D methods in biological research, even in their applications to smaller peptides and proteins, are, among other reasons, caused by the fact that a 2D experiment consists of a single measurement requiring of the order of several days of spectrometer time, whereas the corresponding set of one-dimensional experiments (if it exists at all) may be split up into several measurements requiring spectrometer time of several minutes to several hours (and for this reason may be done with different samples). Therefore, there exist at least three inter-related conditions for 2D experiments to be performed and which are often major obstacles if one wants to apply these methods to enzymes: firstly, the sample must be concentrated to an extent which is not always possible for enzymes; secondly, the sample must be stable under NMR-conditions for several days; thirdly, the NMR-spectrometer has to be available for several successive days. Especially for the last reason, it is of even more importance in 2D spectroscopy than in 1D spectroscopy to use the type of experiment which yields the desired information in the shortest possible time. Two experiments which are clear alternatives to each other in

proton NMR because they aim at the same sort of information are COSY [1] and double-quantum (DQ) [2] experiments, the latter one being the INADEQUATE [3] experiment developed originally for the detection of ¹³C–¹³C connectives in ¹³C-NMR adopted to ¹H-NMR.

COSY is the most primitive 2D-NMR method as far as its pulse sequence is concerned; it basically yields information on directly spin-spin coupled nuclei. Double-Quantum spectroscopy, in contrast, is one of the more advanced 2D techniques, which also gives information on connectivities within spin systems, but only for a rather narrow range of *J*-values, which may be determined by preselection of a delay time within the pulse sequence. The COSY diagonal, which is in principle a representation of the one-dimensional NMR-spectrum, is missing in the DQ-spectrum, so that it is expected that cross-peaks between closely spaced resonances, which may be obscured by the strong diagonal in COSY spectra, are still observable in double-quantum spectra. DQ-spectroscopy seems to offer the additional advantage that remote connectivities can be detected [4] under appropriate conditions such as excellent signal-to-noise ratio.

The DQ experiment has been applied recently to the study of bovine pancreatic trypsin inhibitor [4] as well as to a study of free amino acids with aromatic side chains as compared to the aromatic spectral part of hen egg white lysozyme [5].

Whereas in BPTI (molecular weight: 6500) the vast majority of the proton resonances in the spectrum have been assigned by now to specific amino acid residues in the sequence, ambitions tend to become more modest as the molecular weights of

Reprint requests to Dr. P. Rösch.

0341-0382/84/0700-0738 \$01.30/0

the proteins under investigation increase. In the course of our proton NMR studies on the porcine protein adenylate kinase (AK), which is an ATP:AMP phosphotransferase (molecular weight: 21 500) of known sequence [6] and X-ray structure [7], we have tried to assign at least a few of the aromatic resonances. To this end, and to obtain a comparison between the two methods, we have performed COSY as well as DQ experiments under exactly the same conditions, *i.e.* using the same sample, the same spectrometer settings, and the same amount of spectrometer time.

For our experiments we used a custom-made Bruker 360 CXP NMR-spectrometer equipped with an Aspect-2000 data system and a CDC disk storage system 80/16 Mb. The protein concentration was 2.5 mM at pH 7 in a 10 mm OD sample tube, 2 ml total volume. Amide protons were preexchanged against D₂O for several hours at room temperature. The sample was kept at 25 °C in the magnet. Standard Bruker software was used throughout. For the COSY as well as the DQ experiment a time domain data matrix of size 1024 × 256, developed on a 512 × 512 frequency domain matrix after zero-filling once in f_1 direction, was used. This resulted in a final digital resolution of 6 Hz/point for the COSY matrix in either dimension and 6 Hz/point for the DQ matrix in f_2 dimension and 12 Hz/point

in the f_1 dimension. The COSY-spectrum was acquired with the original Jeener pulse sequence [1] $90^\circ - t_1 - 90^\circ - \text{FID}(t_2)$, the DQ-spectrum with the standard INADEQUATE pulse sequence [3] $90^\circ - t - 180^\circ - t - 90^\circ - t_1 - 90^\circ - \text{FID}(t_2)$, the $1/4J$ delay being optimized for J -couplings of 8 Hertz. The phase cycling scheme described in [3] with a superimposed CYCLOPS phase cycling [8] was used. 624 scans per time increment were collected for the COSY experiment, 640 scans per time increment for the DQ-spectrum, the difference being due to the different phase cycling in the two types of experiment. The relaxation time delay between two pulse sequences was chosen to be 2 s minimum. These parameters resulted in a total measuring time of 90 h per experiment. Quadrature detection in both dimensions was used in both experiments [9, 10]. For signal-to-noise enhancement a sine-bell multiplication was performed in the f_1 direction and a sine-bell-squared multiplication in the f_2 direction in both experiments. The COSY-spectrum was symmetrized with respect to the diagonal [11].

Fig. 1 shows the aromatic part of the adenylate kinase spectrum, consisting of resonances of 2 histidyl, 5 phenylalanyl, and 7 tyrosyl residues (imidazol C2-H not shown). None of the spin connectivities can be guessed immediately.

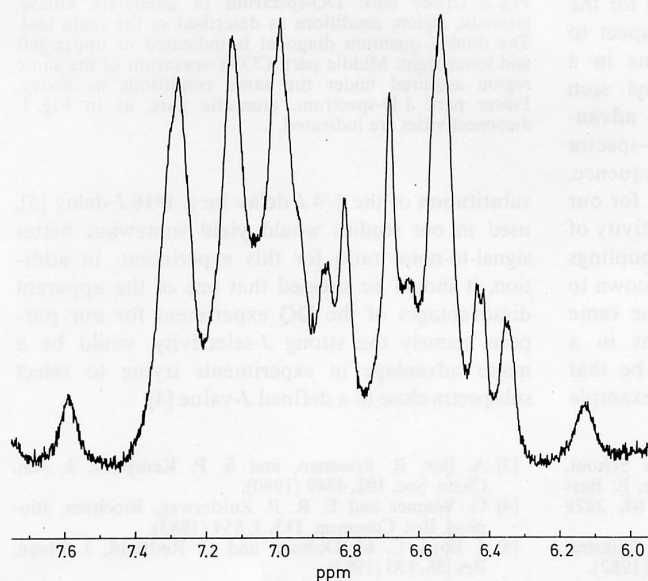


Fig. 1. The ¹H-NMR spectrum of porcine adenylate kinase. pH 7, 0.5 mM protein, 50 mM Tris, $T = 298$ K, 500 accumulations.

Fig. 2 shows, in its upper section, the aromatic part of the DQ-spectrum, the DQ diagonal running from lower right to upper left. (The ppm-scale is referenced to TSP and the carrier frequency – 4.26 ppm downfield from TSP – for the f_2 and the f_1 axes, respectively.) Clearly resolved are 5 connectivities, which, according to their pattern, may with some care be assigned immediately to tyrosyl spin systems [5]. Independent of whether these assignments are correct or incorrect, it can be stated that not all of the seven tyrosyl residues with their at least seven different related DQ connectivities are observable.

The middle section of Fig. 2 shows the same spectral part of the COSY-spectrum as the upper section. Indicated in the figure are the connectivities of nine spin systems, each of which could on first sight be assigned to either a tyrosyl or (part of) a phenylalanyl spin system. In particular, as indicated in the figure, all connectivities found in the DQ-spectrum can also be found in the COSY-spectrum, though, not surprisingly, with a different intensity distribution. The connectivities detected in the DQ-spectrum are therefore a subset of the connectivities detected in the COSY-spectrum.

The lower part of Fig. 2 finally shows the connectivities in the 1D-spectrum as deduced from the connectivities in the COSY-spectrum.

As far as the sensitivity problem is concerned, this all leads to the suspicion that – at least for the purpose of obtaining some clarity with respect to the connectivities in aromatic spin systems in a protein, especially connectivities of tyrosyl spin systems – COSY-spectra offer a far more advantageous signal-to-noise ratio than the DQ-spectra with the original INADEQUATE pulse sequence. The major drawback of the DQ experiment for our current purpose may well be the strong selectivity of this type of experiment concerning the J -couplings of connected spin systems. J -couplings are known to vary by an appreciable amount between the same type of amino acid in different positions in a protein. On the other hand it might well be that some modification of the DQ scheme, for example

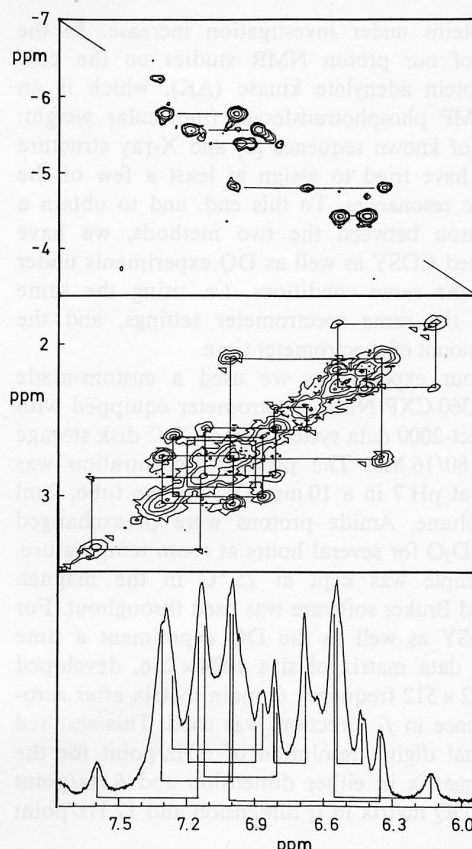


Fig. 2. Upper part: DQ-spectrum of adenylate kinase, aromatic region; conditions as described in the main text. The double quantum diagonal is indicated at upper left and lower right. Middle part: COSY-spectrum of the same region acquired under the same conditions as above. Lower part: 1D-spectrum, aromatic part, as in Fig. 1. J -connectivities are indicated.

substitution of the $1/4J$ delay by a $1/16J$ delay [5], used in our studies would yield somewhat better signal-to-noise ratio for this experiment. In addition, it should be stressed that one of the apparent disadvantages of the DQ experiment for our purpose, namely the strong J -selectivity, could be a major advantage in experiments trying to select subspectra close to a defined J -value [4].

- [1] a) J. Jeener, Ampere International Summer School, Bosko Polje, Yugoslavia 1971; b) W. P. Aue, E. Bartholdi, and R. R. Ernst, J. Chem. Phys. **64**, 2229 (1976).
- [2] P. J. Hore, E. R. P. Zuiderweg, K. Nicolay, K. Dijkstra, and R. Kaptein, J. Am. Chem. Soc. **104**, 4286 (1982).

- [3] A. Bax, R. Freeman, and S. P. Kempsell, J. Am. Chem. Soc. **102**, 4849 (1980).
- [4] G. Wagner and E. R. P. Zuiderweg, Biochem. Biophys. Res. Commun. **113**, 3, 854 (1983).
- [5] J. Boyd, C. M. Dobson, and C. Redfield, J. Magn. Res. **55**, 170 (1983).

- [6] A. Heil, G. Muller, L. Noda, T. Pinder, R. H. Schirmer, I. Schirmer, and I. von Zabern, *Eur. J. Biochem.* **43**, 131 (1974).
- [7] G. E. Schulz, M. Elzinga, F. Marx, and R. H. Schirmer, *Nature* **250**, 120 (1974). *J. Magn. Res.* **43**, 478 (1981).
- [8] D. I. Hoult and R. E. Richards, *Proc. Roy. Soc. Sec. A* **344**, 311 (1975).
- [9] A. Bax, R. Freeman, T. A. Frenkiel, and M. H. Levitt, *J. Magn. Res.* **43**, 478 (1981).
- [10] A. Bax, R. Freeman, and G. Morris, *J. Magn. Res.* **42**, 164 (1981).
- [11] R. Baumann, G. Wider, R. R. Ernst, and K. Wuthrich, *J. Magn. Res.* **44**, 402 (1981).