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Two-dimensional nuclear Overhauser effect in biomolecules

ANIL KUMAR

Department of Physics, Indian Institute of Science, Bangalore 560012, India

Abstract. During the last 5 years, since its first application to biomolecules, two-dimensional nuclear Overhauser effect (2D NOE) has become an extremely powerful technique for assignment of NMR spectra and for elucidation of conformation of biomolecules in solution.

The methodology of the 2D NOE technique, its recent developments and its applications to proteins and oligonucleotides are briefly reviewed.

Keywords. Nuclear Overhauser effect; two-dimensional NMR; peptides and oligonucleotides—structure determination.

1. Introduction

Nuclear Overhauser Effect (NOE), in which non-equilibrium magnetization of spins migrates from one spin to another *via* mutual dipole-dipole relaxation, is a powerful tool for structural elucidation of molecules in liquid state. One-dimensional difference NOE experiments performed by selective saturation or inversion of a resonance have provided a rich source of structural information in biomolecules, but require a large number of selective experiments. Application of two-dimensional (2D) techniques for monitoring NOE in biomolecules had a revolutionary effect in the study of biomolecular structures by NMR.

The scheme for 2D NOE experiments first proposed for chemical exchange by Jeener (1977) and exploited mainly for study of chemical exchange by Meier and Ernst (1979) and Jeener *et al* (1979) is given in figure 1. Non-equilibrium z-magnetization of all spins produced by the second 90° pulse after frequency labelling during t_1, undergoes dipole-dipole relaxation and chemical exchange, if any, during a fixed mixing period τ_m at the end of which the third 90° pulse reads the state of each spin as a function of t_2. The rate equations governing this transfer during τ_m (Jeener *et al* 1979) are similar to those of 1D transient NOE experiments (Solomon 1955; Kalk and Berendsen 1976).

The first 2D NOE spectrum of a biomolecule (Anil Kumar *et al* 1980a) is shown in figure 2. The numerous cross-peaks produced in this spectrum provide a rich source of information on the proximity of various protons in the biomolecule. Biomolecules which reorient slowly on the NMR time-scale such that $\omega\tau_c \gg 1$ where ω is the resonance frequency and τ_c is the characteristic reorientation time, known as correlation time of the molecule, give negative NOE and favourable cross-peak intensity in the 2D NOE experiment (Macura and Ernst 1980). Small molecules which reorient faster, for which $\omega\tau_c \ll 1$ and give positive NOE, yield poor cross-peak intensity in the 2D NOE experiment. Molecules for which $\omega\tau_c \sim 1$ give no NOE. The effects of J coupling on the 2D NOE experiment have been studied (Macura *et al* 1981, 1982). Details of the experimental procedure and parameters required for proton 2D experiments in proteins including details of phase cycling employed have been described (Wider *et al* 1984).

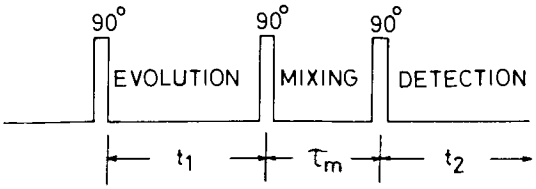

Figure 1. Pulse scheme for 2D NOE experiment. Signal is collected during t_2, for a complete set of t_1 values and for a fixed interval τ_m during which nuclear Overhauser effect takes place. The phase cycling for cancellation of axial peaks and transverse magnetization after the second pulse and for quadrature detection in both dimensions has been described (Wider *et al* 1984).

2. Methodology of 2D NOE

The build-up rate of 2D NOE has been studied as a function of mixing time τ_m (Anil Kumar *et al* 1981). In biomolecules the spin-diffusion tends to make the NOE information non-specific and experiments with controlled mixing time have been suggested to overcome this problem in 1D NOE schemes (Krishna *et al* 1978; Gordon and Wuthrich 1978; Dubs *et al* 1979). Furthermore, theoretically it has been shown that the initial build-up rate of 2D NOE is directly proportional to r^{-6}, where r is the distance to the nearest proton from the proton of interest (Macura and Ernst 1980). While factors such as differences in linewidths, relaxation times, multiplicity of coupling partners and effects of filter functions make it difficult to use the 2D NOE information quantitatively, attempts are underway to utilize the 2D NOE data for quantitative distance measurements (Wagner *et al* 1984; Keepers and James 1984; James 1984). So far, however, distance information from 2D NOE has been effectively utilized in qualitative or at best in semi-quantitative manner as upper and lower bounds on distances.

Another important feature of 2D NOE and 2D correlated (COSY)* experiments is the ability to perform these experiments in H_2O solutions and obtain information on exchangeable protons (Anil Kumar *et al* 1980b; Wider *et al* 1983). This has been a key development in obtaining complete assignments and structural information on biomolecules. For suppression of H_2O signal in 2D NOE experiment three schemes have been suggested. The most widely used scheme utilizes selective saturation of H_2O resonance by irradiation (Anil Kumar *et al* 1980b; Wider *et al* 1983). Cutnell (1982) suggested replacement of last 90° pulse by 2-1-4 sequence (Redfield *et al* 1975) with a null at H_2O resonance position in its excitation profile. Another technique utilizes the observation that the T_1 of H_2O protons is much longer than those of protein resonances. A non-selective inversion by a 180° pulse followed by a recovery period $= \tau_{null}$ for H_2O resonance, precedes the 2D NOE experiment, accomplishing effective suppression of H_2O signal, almost full recovery of protein signals and unmasking of protein resonances buried under the H_2O signal (Basus 1984).

2D NOE experiment is utilized in an interactive manner with COSY for spectral

*Off diagonal peaks in COSY indicate spin-spin coupled protons and is a powerful technique for resonance assignment of biomolecules (Anil Kumar *et al* 1980b; Wagner *et al* 1981).

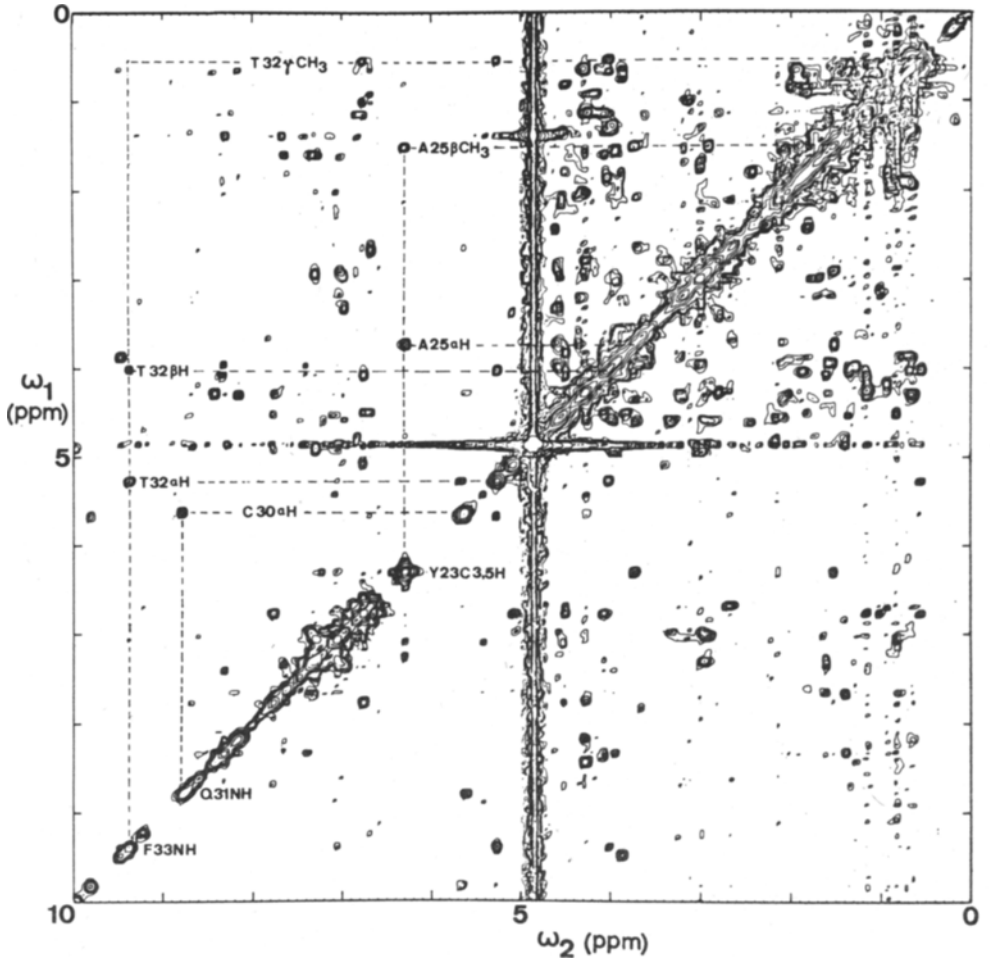

Figure 2. 2D NOE spectrum of basic pancreatic trypsin inhibitor (BPTI) recorded at 360 MHz and τ_m = 100 m sec. The protein concentration was 20 mM in D_2O, at pH = 3·8 and T = 18°C. The spectral width = 4000 Hz and 512 data points were collected in each dimension, with total data accumulation time of 18 hr. Shifted sine bell filtering has been utilized in both dimensions and an absolute intensity contour plot is shown here. Some of the β-sheet NOE's; those between phenylalanine NH proton (F33NH) and threonine (T) C^α, C^β and C^γ protons, between glutamine (Q) NH proton and cysteine (C) C^α proton and between tyrosine (Y) ring protons and alanine (A) C^α and C^β protons are indicated by broken lines. Subsequent analysis of this data (Wagner *et al* 1981), and additional data (Wagner and Wüthrich 1982) have yielded essentially complete assignment of all backbone and C^β proton resonances (from Anil Kumar *et al* 1980a).

assignment and sequence determination. Combined with one triangle of COSY data and other triangle of NOE data, diagrams such as COSY-NOESY have yielded features which are used for tracking down secondary structures such as β-sheet in biomolecules (Wagner *et al* 1981). This is due to the special feature of β structure in which the amide proton of an aminoacid residue lies close to the $C^\alpha H$ proton of the preceding residue. Starting

from a known NH proton resonance, NOE gives strong cross-peak to preceding $C^{\alpha}H$ proton, which in turn has cross-peak with NH proton of the same residue in the COSY. Continuing from the NH proton the resonance assignments of the backbone resonances of the entire β-secondary structure can be obtained (Wagner *et al* 1981).

3. Applications to proteins

The information from 2D NOE has been effectively utilized by Wüthrich and coworkers for structure determination of biomolecules. The NOE between NH proton of adjacent aminoacid residues of a polypeptide chain has been called d_2-connectivity, while those between NH proton and $C^{\alpha}H$ and $C^{\beta}H$ protons of preceding residue respectively as d_1 and d_3 connectivity (Billeter *et al* 1982). Sequential resonance assignments have been obtained in the backbone region of several small proteins, by careful and large scale application of 2D techniques, the strategy for which is summarized in Wüthrich *et al* (1982) and Wüthrich (1983). Essentially complete resonance assignments have been obtained for backbone and $C^{\beta}H$ proton resonances of basic pancreatic trypsin inhibitor (BPTI), a globular protein of 56 aminoacid residues (Wagner and Wüthrich 1982a), glucagon bound to perdeuterated micelles (Wider *et al* 1982), trypsin inhibitor E (Arseniev *et al* 1982), proteinase inhibitors IIA and IIB from Bull Seminal Plasma (BUSI IIA and IIB) (Strop *et al* 1983a, b) and cardiotoxin V^{II_2} (Steinmetz *et al* 1981 and Hosur *et al* 1983).

Distance geometry algorithms have been written with aims of obtaining three-dimensional conformation of small proteins with distance constraints as obtained from 2D NOE data (Braun *et al* 1981; Havel and Wüthrich 1984a, b). The new distance geometry program DISGOE (Havel and Wüthrich 1984) capable of computing complete spatial structures of polypeptide chains upto *ca.* 100 aminoacid residues, has been tested for its ability to compute structures of biomolecules from 2D NOE data, by comparing the calculated structure of BPTI to its known crystal structure (Havel and Wüthrich 1985). Using the resonance assignments mentioned above, the distance information as obtained from 2D NOE and the distance geometry algorithms, three-dimensional structures have been calculated for glucagon (Braun *et al* 1983), BUSI IIA (Williamson *et al* 1984), BPTI (Wagner *et al* 1984) and portions of micelle-bound melittin (Brown *et al* 1982). The strategies utilized for the above structure determination have been outlined by Wüthrich *et al* (1983, 1984).

Various 2D techniques including 2D NOE have been utilized for studying the solution structure of Alamethicin (Banerjee *et al* 1983) and gramicidin A double helix (Arseniev *et al* 1984a). Arseniev *et al* (1984b) have also studied the three-dimensional solution structure of a short insectotoxin I_5A using various 2D NMR techniques including 2D NOE, have utilized the distance geometry algorithm of Braun *et al* (1981) and found similarity with the known single crystal α-helical and antiparallel β-structure of a homologous 'long' toxin v-3. Wemmer and Kallenbach (1983) studied the 18 aminoacid residue neurotoxin apamin, obtained essentially complete resonance assignments using COSY and 2D NOE, and obtained evidence for secondary structures consisting of a β turn and a α-helix.

Zuiderweg *et al* (1983, 1984a, b) have studied the structure of the headpiece of LAC repressor, residue 1-51, and found that the headpiece contains three α-helices connected by regions of less regular structure.

4. Applications to nucleotides/saccharides

2D NOE experiment has been effectively utilized by several groups for resonance assignments in oligonucleotides along with COSY experiments. Usually these DNA fragments are in some well-defined helical conformation such as B-DNA and a few typical NOEs are able to confirm such a configuration. The remaining NOEs in the molecule are then used for resonance assignment and conformation determination in an interactive manner.

Hare *et al* (1983) studied self-complementary DNA sequence d(CGCGAATTCGCG) by the above 2D techniques, obtained complete resonance assignments and from the observed NOE data concluded that in solution this helix is right-handed close to the B-DNA form in conformity with the crystallographic structure. Scheek *et al* (1984) used this algorithm to obtain resonance assignments in a mixture of two synthetic complementary heptamers d(TGAGCGG) and d(CCGCTCA) forming a duplex. Broido *et al* (1984) used 500 MHz, phase-sensitive 2D NOE experiments performed with several mixing times to obtain complete resonance assignments in self-complementary octamer duplex $[d\text{-}(GGAATTCC)]_2$, the largest oligonucleotide yet assigned. Weiss *et al* (1984a,b) carried out assignments of major groove sugar protons of the 17 base pair DNA operator site O_L1 by 2D NOE and COSY. Haasnoot *et al* (1983) have done sequential assignment and conformational analysis of d(CG) r(CG) d(CG) using COSY and 2D NOE.

Feigon *et al* (1982, 1983) have used 2D NOE along with COSY and 1D experiments for obtaining complete proton resonance assignments in double stranded synthetic DNA decamer d(ATATCGATAT). The observed NOEs further establish that the nucleotides have an anti-conformation of the bases relative to sugar which is consistent with the B-form of DNA. Broido and Kearns (1982), on the other hand, have used 2D NOE and other relaxation measurements to conclude that poly C forms a left-handed helical structure in neutral solution.

Hilbers *et al* (1983) have studied the solution structure of yeast $tRNA^{phe}$ using 2D NOE, have totally assigned imino proton spectrum, and have shown that the principal elements of x-ray structure of tRNA *i.e.* the hydrogen bonding network and the stacking of the stems upon one another, are also found in solution. The imino protons of several pure *E. coli* isoacceptor tRNA species have been assigned by 2D NOE in H_2O solution (Reid *et al* 1984). Borah *et al* (1984) have utilized phase-sensitive 2D NOE spectra, for studying three-dimensional conformation of several sonicated poly-deoxynucleotides in solution. They find that poly(dAdT)·poly(dAdT) and poly(dGm^{3}dc)·poly(dGm^{3}dc) in low salt and poly(dAdT)·poly(dAdT) in high salt are right-handed B-structures, in contrast to suggestions that poly(dAdT)·poly(dAdT) exists as a left-handed form either in low or high salt. Ravikumar *et al* (1984) used 2D methods, including 2D NOE, for studying the solution structure of $d(CG)_6$ in low salt concentration and found indications of B-DNA structure, at least in some parts of the molecule.

Clore and Gronenborn (1985) have recently reviewed the use of 1D and 2D NOE for three-dimensional structure determination of DNA and RNA oligonucleotides in solution and have studied the solution structure of DNA hexamer d(CGTACG) and octamer d(ACGCGCGT) (Clore and Gronenborn 1984; Clore *et al* 1985).

2D NOE has been utilised for determination of sequence and linkage sites in ceramide trisaccharide (Prestegard *et al* 1982) and for sequence and linkage site determination in

oligosaccharides of gangliosides (Koerner *et al* 1983). Homans *et al* (1984) have used 2D NOE along with other 1D and 2D NMR techniques for structural and conformational analysis of oligosaccharides.

5. Other developments

5.1 *Accordian 2D NOE spectroscopy*

Bodenhausen and Ernst (1981, 1982) extended the 2D NOE experiment into third time dimension without increasing the experimental time. The mixing time τ_m of 2D NOE scheme, figure 1, was incremented in concert with t_1, such that $\tau_m = kt_1$ where k is a constant, and appropriately named this scheme as Accordian spectroscopy. The lineshapes of the cross and diagonal peaks of the resulting 2D NOE spectrum contain information on the rate constants governing the growth and decay of these peaks. It was shown that these rate constants could be extracted, in a straightforward manner, by appropriate data handling. However, whenever lines are overlapped or have multiplet structure as happens rather often in proton NMR spectra of biomolecules, the analysis of the data becomes complex. So far, application of Accordian spectroscopy has been limited to study chemical exchange networks *via* carbon-13 experiments where non-overlapping resonances are obtained (Huang *et al* 1981; Bodenhausen and Ernst 1982).

5.2 *Combined COSY/NOESY experiments*

Recently two groups have independently suggested collection of data following both the second and third pulses in the 2D NOE scheme, thus making it possible to collect both COSY and NOESY data in a single experiment, with the resolution in ω_2 dimension for COSY limited to $1/(2\tau_m)$ (Gurevich *et al* 1984; Haasnoot *et al* 1984). Appropriate phase cycling for carrying out these experiments have been given.

5.3 *Relayed coherence transfer 2D NOE*

Wagner (1984) has suggested combining a coherent transfer step along with 2D NOE experiment either before the NOE transfer or after, and named it Relayed NOESY. These experiments have been suggested to transfer a NOE peak from crowded to sparse spectral region and to solve ambiguities in the interpretation of NOESY cross-peaks.

5.4 *CIDNP and 2D*

Scheek *et al* (1984) have combined photochemically-induced dynamic nuclear polarization (photo-CIDNP) and 2D techniques (COSY, NOESY and INEPT) for identifying selectively three aromatic amino acid residues (tryptophan, tyrosine and histidine) on the surfaces of proteins. The 2D techniques are preceded by a saturation pulse and a short laser pulse to generate CIDNP. Interesting 2D spectra which lack the symmetry of conventional 2D spectra have been obtained in biomolecules.

6. Conclusions

The 2D NOE experiment has provided key information on conformation, dynamical properties and assignment of resonances of biomolecules which would have been

difficult or impossible to obtain from conventional NMR methods. In addition it has provided large number of distance constraints making it possible to compute three-dimensional structures of polypeptide chains in aqueous solution, making it one of the most powerful 2D technique for biological systems.

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