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CORRELATION NMR SPECTROSCOPY AND ITS APPLICATIONS

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Abstract - A correlation NMR system has been described. Several problems which are inherent in implementing the correlation technique on a mini-computer are discussed, and some examples of application are given.

Since first introduced by Dadok(1), correlation NMR has been recognized as a powerful technique for the investigation of biological systems which always contain a large amount of water. Only a portion of the spectrum is scanned in correlation NMR, and therefore dynamic range problems which are frequently encountered in dealing with biological systems with pulse FT NMR are much less serious. In the present paper, several problems which are inherent in implementing correlation ^{1}H NMR on a mini-computer will be discussed. Some applications of this technique will also be described.

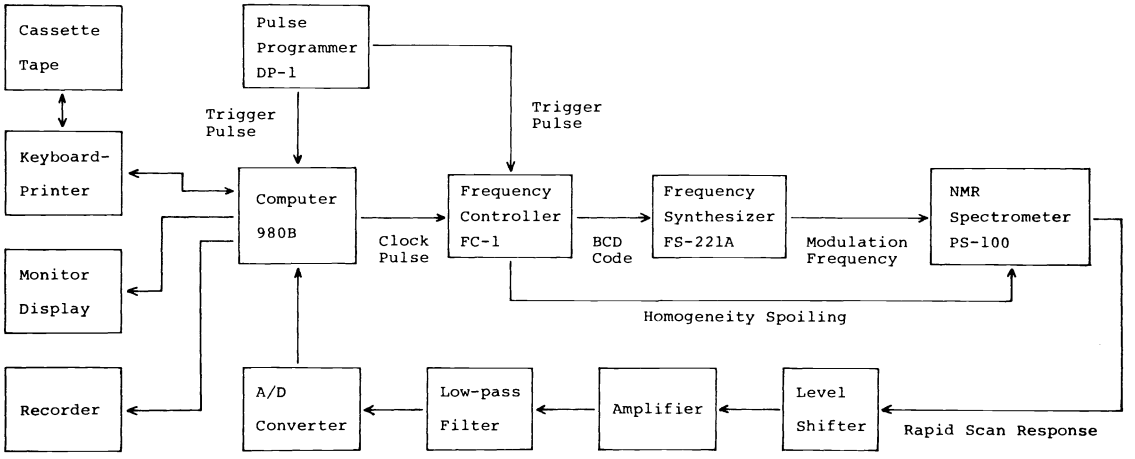

Fig.1. Block diagram of correlation NMR system

A JEOL PS-100 NMR spectrometer operating at 100 MHz was used in conjunction with a Texas Instruments 980B computer with 32K words of memory(16 bits/word). Modulation frequency at about 8 kHz is swept linearly by using a TOA FS-221A frequency synthesizer which is driven by a series of BCD codes from a frequency controller which is controlled by the clock pulse from the computer. A JEOL pulse programmer DP-1 is used to control the computer and the frequency controller. The frequency controller is also used to short-circuit a part of the shim coils to destroy any residual transverse magnetization after a linear frequency sweep. A crystal filter is placed in the i.f.stage of the receiver to improve the signal to noise ratio. Transient response to the frequency sweep is passed through a four-pole Butterworth filter, and collected on the computer through an analog-to-digital converter(ADC) with a resolution of 12 bits. Accumulated transient data are processed using a program written on the basis of a slightly modified version of the theoretical function method described by Gupta, Ferretti, and Becker(2).

Several features of the present system and some of its application are summarized in the following.

1.Floating-point arithmetic
Since one word is 16 bits on the 980B computer, and the resolution of the ADC is 12 bits, stored transient data may overflow after more than 16 accumulations. In the present system, transient response data are converted into floating-point number before memory overflows, and transferred to other part of memory, where acquired data are stored, and accumulated in floating-point number as accumulation continues. Acquired data are then processed in floating-point arithmetic. A long term accumulation can thus be made without either scaling down the acquired data, or performing block averaging such as used in pulse FT NMR. A total computation time of about 100 sec is necessary for the data processing of a 1K rapid scan response in floating-point arithmetic. Another advantage of using floating-point arithmetic is that roundoff errors in data processing involving fast Fourier transformation can be minimized as compared to a conventional fixed-point FFT algorithm which is commonly used in a commercial FT NMR unit(3). Statistical analysis of roundoff errors in the data processing of correlation NMR will be described elsewhere.

2.Tilted baseline problem
In correlation NMR, one usually deals with small signals which lie on the shoulder of a huge signal such as coming from the solvent water. However, when the baseline is tilted, a strong oscillation appears as shown in Fig.2a.

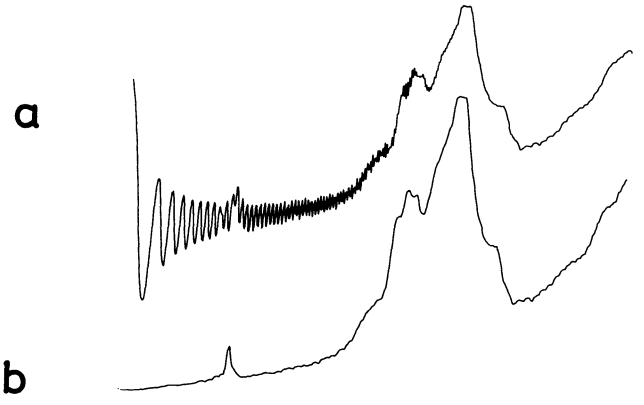

Fig.2. 100 MHz correlation ^{1}H NMR spectra of the aromatic region of hen egg white lysozyme (spectral width: 250 Hz) obtained with
(a) a conventional correlation procedure and
(b) a modified procedure involving a wing processing.

A wing processing incorporated in the present system has been shown to be quite useful in dealing with a spectrum with a tilted baseline. In this method(4), the first and last points of a rapid scan response (N data points) are provided with wings with 3N/8 identical values which are equal to the first and last points, respectively. Another N/8 points are used to apodize both ends of the wings with a quarter cycle of the sine function. The winged rapid scan response with 2N data points thus obtained is processed by the theoretical function method, and the spectrum is cut out of the relevant part of the final result. A mathematical basis of this technique will be described elsewhere. The rapid scan response which gives Fig.2a was treated with this wing processing, and the spectrum obtained is shown in Fig.2b, where the undesirble oscillation of the baseline is clearly eliminated. It should be noted that the procedure described here does not give rise to any distortion of the spectrum such as line broadening. In order to improve the accuracy of subsequent data analysis, a baseline-flattening routine has also been incorporated.

3.Minimum number of sampling points required

The resonance conditions are met sequentially in correlation NMR spectroscopy. Therefore, if only a portion of the spectrum is scanned, the number of sampling points can be made very small without violating the sampling theorem. The final spectrum obtained using the minimum number of sampling points required may not be smooth enough, making it difficult to find the peak maxima. However, interpolation can easily be made by supplementing a sufficient number of zeros at the end of the free induction decay which is derived from the rapid scan response, in a similar way as reported by Bartholdi and Ernst for pulse FT NMR(5). In correlation NMR, this feature should be especially important when one wants to observe, for example, carbon-13 spectra where signals generally scatter over a wide range of frequency. Fig.3a shows a part of the aromatic region of a ^1H NMR spectrum of hen egg white lysozyme where a total spectral width of 516 Hz was covered with 128 data points. The same region of the spectrum obtained by supplementing 128 and 128 x 7 zeros at the end of the free induction decay is given in Figs.3b and 3c, respectively. The spectrum shown in Fig.3c is quite similar (with the exception of high-

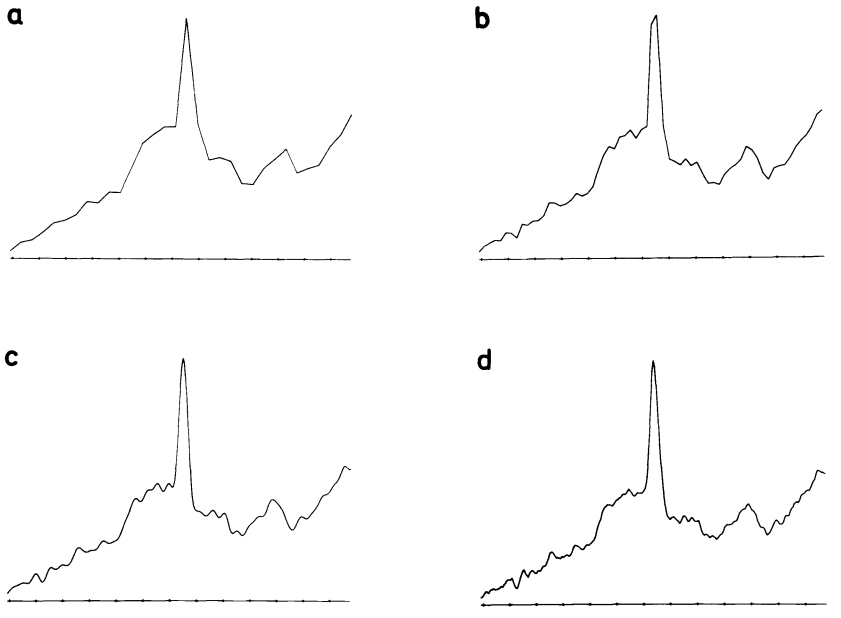

Fig.3. 100 MHz correlation ^1H NMR spectra of the C2-H proton of histidine of hen egg white lysozyme in D_2O
(a) obtained from a rapid scan response covering a total spectral width of 516 Hz with 128 data points,
(b) and (c) derived from the free induction decay used to obtain the spectrum (a), by supplementing 128 and 128 x 7 zeros at is end, respectively, and
(d) obtained from a rapid scan response covering a total spectral width of 516 Hz with 1024 data points.
One division is equal to 10 Hz.

frequency noise) to that presented in Fig.3d which is obtained from a rapid scan response with 1024 data points.

4.Measurements of spin-lattice relaxation times

A set of two frequency sweeps passing through resonance lines of interest is used to measure spin-lattice relaxation times by adiabatic rapid passage method(6). In sweep 1 there is employed rf power which is sufficiently high to meet the condition of adiabatic rapid passage for a given sweep rate. After a time of τ, sweep 2 is initiated at a weaker rf level to acquire partially relaxed transient data. The sequence of the two sweeps is equivalent to the 180°-τ-90° sequence in pulse FT NMR. Any residual transverse magnetization after adiabatic inversion is eliminated, if necessary, by homogeneity spoiling. Figure 4 shows a series of partially relaxed fast passage

transients and correlation NMR spectra of a mixture of dichloromethane,

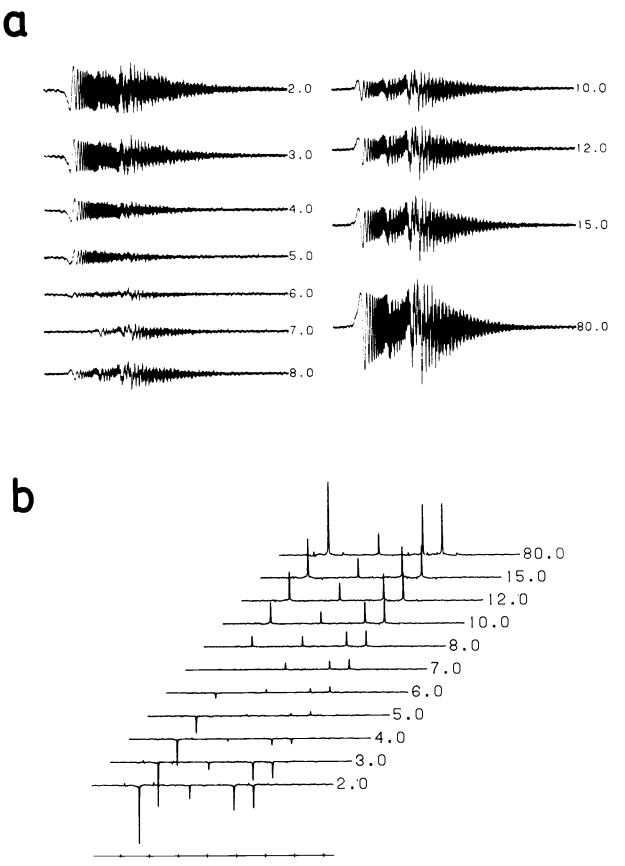

Fig.4. Partially relaxed rapid scan responses(a) and the corresponding correlation NMR spectra(b) of a mixture of dichloromethane, acetone, dioxane, and cyclohexane (from low to high field). Each transient was sampled after adiabatic inversion with a sweep interval τ (in sec) shown in the figure. One division is equal to 100 Hz. Sweep rate: 1634 Hz/sec, Sampling time: 1.024 sec.

acetone, dioxane, and cyclohexane taken with different τ's after adiabatic inversion. The same sample was used to determine T_1's using the 180°-τ-90° sequence by pulse FT NMR. The T_1 values obtained by these two methods are in good agreement with each other. Gupta, Ferretti, and Becker(7) have proposed a saturation method in correlation NMR for the measurement of T_1. In the adiabatic rapid passage method used in the present work, the initial state of the spin system can be defined strictly for each of the resonance lines. Therefore, it appears that the present method is more versatile in dealing with spectra with more than two lines. However, it should be noted that the adiabatic rapid passage method may well be subject to further complications when connected transitions are swept through, in which case partially relaxed correlation NMR spectra have to be interpreted with care. Otherwise, the present technique should be useful for measuring T_1 of the order of 1 sec for a part of a spectrum without perturbing the remaining portion of it.

5.Selective excitation of a weakly coupled non-equilibrium spin system

In pulse FT NMR experiments of non-equilibrium spin states, a sufficiently small flip angle is necessary for the observation of the same relative intensities as obtained in a slow passage low-power experiments(8). In a weakly

coupled spin system, it is in general possible in the correlation mode to cover a part of the spin system, leaving the remaining portion of it intact. In the present work, correlation NMR has been applied to CIDNP in a weakly coupled case(9). A flip angle of approximately 90° was used in the correlation mode to record NMR spectra of a reaction mixture of 4-picoline-N-oxide and acetic anhydride. An example of the spectrum is reproduced in Fig.5.

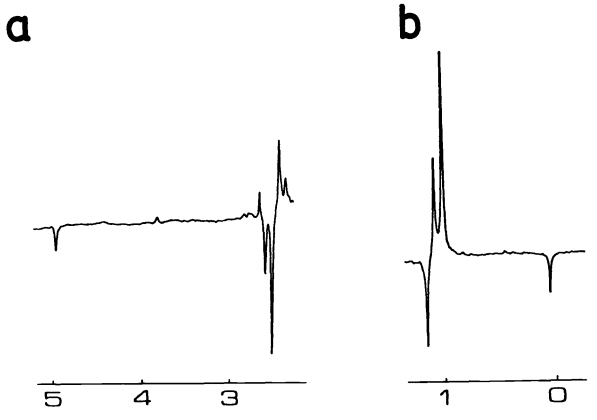

Fig.5. 100 MHz correlation ^{1}H NMR spectrum of a reaction mixture of 4-picoline-N-oxide and acetic anhydride obtained by using a flip angle of approximately 90°. The rapid scan response was recorded at 70 sec after insertion of a sample tube into a probe preheated at 98°.
(a) Sweep is from high to low field. Sweep rate: 205.5 Hz/sec, Sampling time: 2.048 sec.
(b) Sweep is from low to high field. Sweep rate: 170.2 Hz/sec, Sampling time: 2.048 sec.
Chemical shifts are in ppm from internal TMS.

It has been confirmed that the spectral pattern shows no dependence on the flip angle. The spectrum observed in the correlation mode is quite similar to that observed in the CW mode(10). When large flip angles are used in the pulse FT mode, the multiplet effect disappears as predicted(8). The above result should be an obvious advantage of the correlation method over the pulse FT method, which reproduces the correct intensity pattern only when a small flip angle is employed at a sacrifice in intensity.

6.Application to non-invasive analysis of biological systems

High resolution ^{13}C and ^{31}P NMR have been used for non-invasive analysis of a variety of intact tissues and cellular organelles. ^{1}H NMR has the great advantage that the sensitivity is much better than ^{13}C and ^{31}P NMR, and that ^{1}H nuclei exist virtually in all biologically important molecules. However, a large dynamic range for the signal detection is required in using ^{1}H NMR to deal with biological systems where a large amount of water always exist. Correlation ^{1}H NMR is one of most promising techniques for this purpose. The system described in the present paper has several features which are important in applying correlation ^{1}H NMR to biological systems. The wing processing in combination with a baseline flattening routine is quite effective in dealing with small signals from water solutions. Homogeneity spoiling is also necessary in most cases in order to reduce the sweep repetition time. Figure 6 shows an example of the use of the homogeneity spoiling in correlation NMR. A crystal filter was placed in the i.f.stage (at 11 MHz) of the receiver. Figure 7 gives correlation ^{1}H NMR spectra of the C2- and C4-H protons of histidine in H_2O (0.5 mM), taken with and without the crystal filter. Clearly a significant improvement of the signal to noise ratio is made by the introduction of a crystal filter.

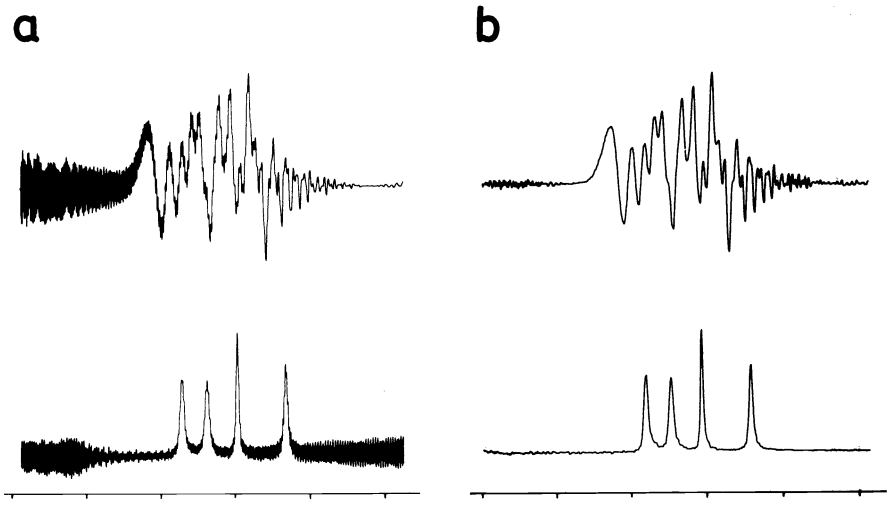

Fig.6. 100 MHz correlation ^1H NMR spectra (lower traces) of tetraglycine in 90% D_2O (10 mM, pH 6.2). Sweep is from high to low field to record rapid scan responses (upper traces), which are displayed with the high field side on the left. Sweep time: 3.0 sec, Sweep repetition time: 3.2 sec, Number of scans: 64.
(a) Homospoiling: off
(b) Homospoiling: on (0.1 sec)
One division is equal to 20 Hz.

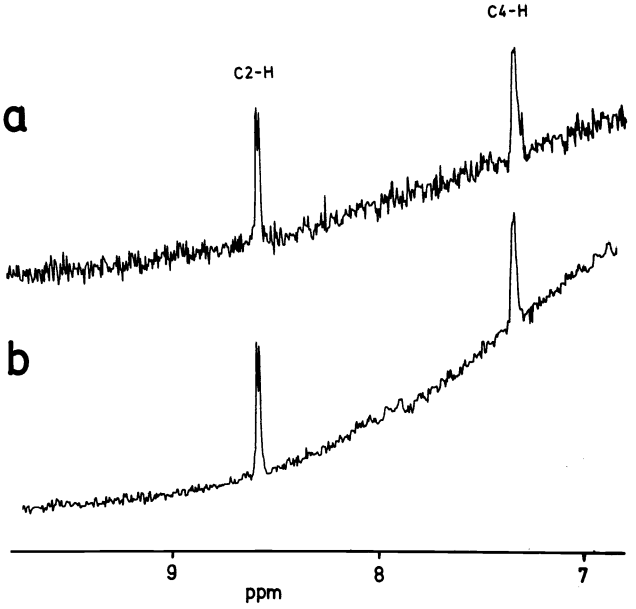

Fig.7. 100 MHz correlation ^1H NMR spectra of the C2- and C4-H protons of histidine in H_2O (0.5 mM, pH 4.5) recorded
(a) without a crystal filter
(b) with a crystal filter placed in the i.f.stage.
Other experimental conditions are the same in the two measurements. Sweep time: 3.0 sec, Sweep repetition time: 3.2 sec, Homospoiling: 0.1 sec, Number of scans: 512.

Figure 8 shows ^{1}H NMR spectra of a suspension of E.coli grown anaerobically using glucose as sole carbon source. Ethanol, lactate, acetate, pyruvate,

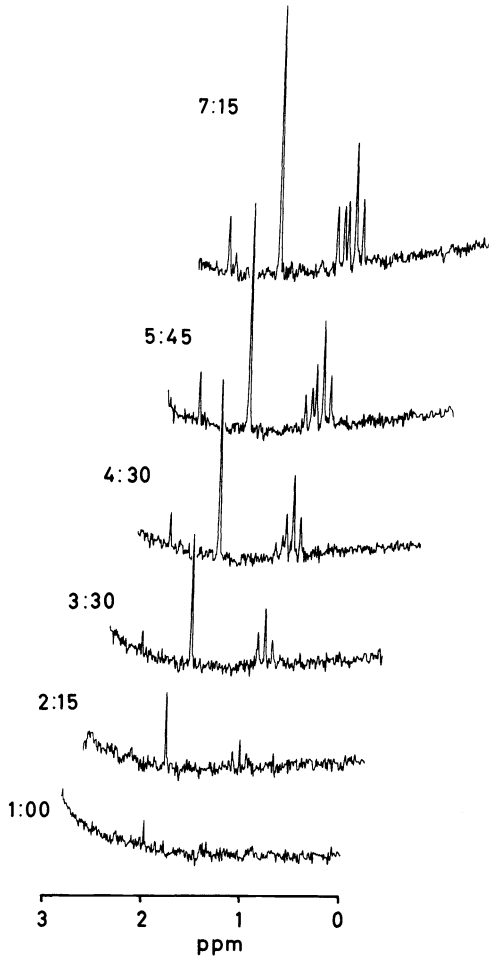

Fig.8. Anaerobic fermentation by E.coli as followed by correlation ^{1}H NMR. Chemical shifts are in ppm from external DSS. E.coli cells grown to the exponential phase in an M9 medium with 0.1 M glucose as sole carbon source were harvested by low-speed centrifugation. The pellet obtained was suspended at a cell density of 0.5×10^{9} in the same medium, and kept in a 5 mm NMR tube, bubbled with nitrogen gas, and the resultant inhomogeneous cell suspension was incubated anaerobically in an NMR probe at 30°. The pH of the medium was adjusted to 7.2 at the beginning of the growth. In the figure, incubation times after inoculation are given in hours.

and succinate (from high to low field) can clearly be identified. Metabolites at a concentration of 0.1 mM can easily be detected after accumulation of several minutes. The time course of the concentration of metabolites observed in this and related experiments are now being used to investigate dynamical aspects of the anaerobic metabolism in E.coli cells(11).

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