Tetrahedron Letters,Vol.24,No.5,pp 473-476,1983 0040-4039/83/050473-04$03.00/0
Printed in Great Britain 

# REASSIGNMENTS IN THE $^1$H NMR SPECTRUM OF FLAVIN ADENINE DINUCLEOTIDE BY TWO-DIMENSIONAL HOMONUCLEAR CHEMICAL SHIFT CORRELATION

*Ulrich, Eldon L., Westler, William M., and Markley, John L.
Department of Chemistry, Purdue University, West Lafayette, IN 47907

Abstract: The ribitol $^1$H NMR proton resonances for flavin adenine dinucleotide have been assigned using homonuclear two dimensional NMR spectroscopy.

We have used homonuclear $^1$H chemical shift correlated two dimensional Fourier transform NMR spectroscopy ($^1$H correlated 2DFT) (1,2) to assign the ribitol proton peaks in the 470 MHz $^1$H NMR spectrum of flavin adenine dinucleotide (FAD). A diagram of the FAD structure is displayed in Figure 1 with all of the carbon bound protons labeled. The reported peak assignments for the FAD ribitol protons (3) were not compatible with our initial double resonance experiments. An unambiguous assignment of the ribitol proton peaks was, however, not possible using one-dimensional NMR techniques because of the severe overlap of the resonances. The ribose and ribitol proton region of a normal FAD $^1$H NMR spectrum and the $^1$H correlated 2DFT spectrum are presented in Figure 2. The

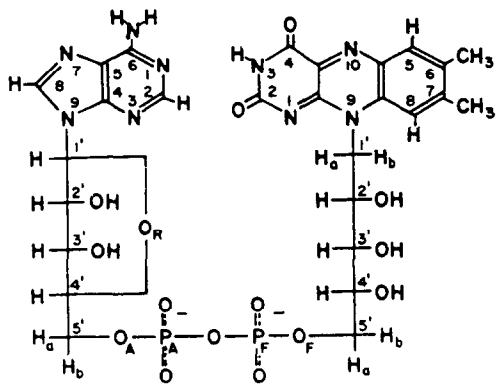

Figure 1 Drawing of the FAD structure

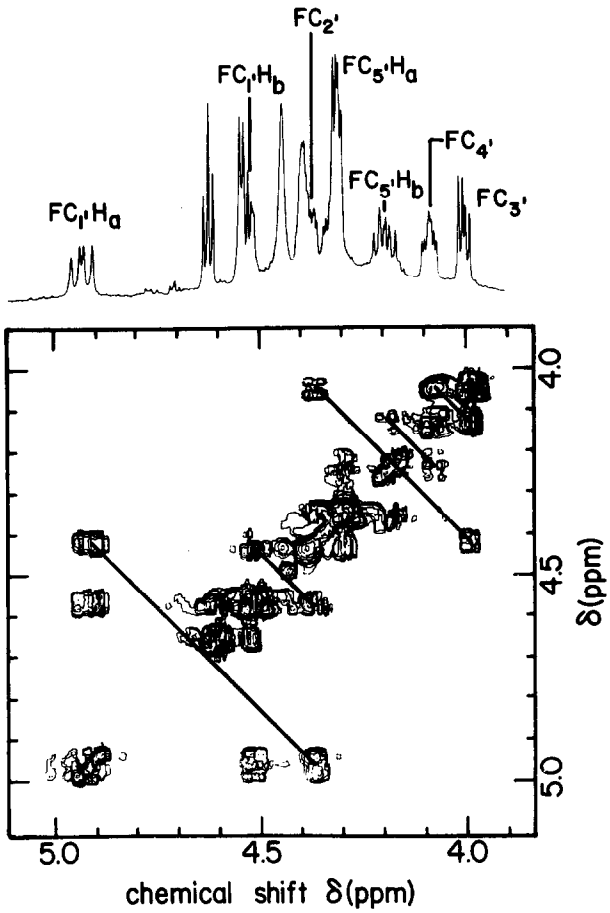


Figure 2: Homonuclear two-dimensional correlated 470 MHz $^{1}H$ NMR spectrum of FAD at 70 °C. Above the correlated spectrum is the one-dimensional spectrum of the same region. The spectrometer sweep width was ± 2000 Hz, and the data were collected using quadrature detection on a Nicolet NT-470 NMR spectrometer. The correlated spectrum was collected using a (90°-τ-60°-acquisition) pulse sequence with the carrier frequency placed at the low field end of the spectrum. The data were collected as a 1024 X 1024 block of data points and the final spectrum was a block of 512 real data points. The variable time τ was incremented from 10 μs to 128.25 ms in steps of 250 μs. Each spectrum corresponding to an individual τ value was the sum of 4 free induction decays. The sample contained 0.1M FAD in $^{2}H_2O$. Only the protons of the ribitol group are labeled. The other resonances arise from the ribose protons in FAD or HDO.

increased resolution afforded by two-dimensional spectroscopy allows for the complete assignment of all the proton peaks in FAD. In a homonuclear correlated 2DFT spectrum there are two classes of peaks. The peaks forming a diagonal across the spectrum are the same as those in normal one-dimensional spectroscopy. The off-diagonal peaks reside at the intersection of the chemical shifts of resonances sharing common energy levels and thus correlate the chemical shifts of protons which are scalar coupled.

In the $^1H$ NMR spectrum of FAD the peaks at 5.93 ppm and 4.92 ppm are assigned to the ribose $C_1'$ proton and one of the ribitol $C_1'$ protons, respectively (3). These assignments are based on comparisons between spectra of FAD, FMN (flavin mononucleotide) and ADP (adenosine diphosphate). From these assignments we have used the pattern of spin coupled resonances shown in Figure 2 to assign the FAD ribose and ribitol peaks. A 1024 by 1024 data set was used to assure the ability to resolve coupling constants greater than 1 Hz. Our assignments for the ribose ring protons agree with those reported previously (3). The FAD ribitol proton chemical shifts are listed in Table I.

Table I: Chemical Shifts and Coupling Constants for FAD Ribitol Protons at 70 °C

| | $\delta^{\dagger}$ | | $J(Hz)^{\dagger}$ |
|---|---|---|---|
| $FC_1'H_a$ | 4.92 | $F1_a'1_b'$ | 14.2 |
| $FC_1'H_b$ | 4.52 | $F1_a'2'$ | 9.8 |
| $FC_2'$ | 4.36 | $F1_b'2'$ | 2.5 |
| $FC_3'$ | 3.99 | $F2'3'$ | 4.7 |
| $FC_4'$ | 4.08 | $F3'4'$ | 7.6 |
| $FC_5'H_a$ | $4.3^{\dagger\dagger}$ | $F4'5_a'$ | 2.9 |
| $FC_5'H_b$ | 4.18 | $F4'5_b'$ | 5.9 |

$^{\dagger}$Determined from a one dimensional $^1H$ NMR spectrum of FAD at 470 MHz

$^{\dagger\dagger}$Can not be determined accurately due to overlapping ribose peaks.

The ribitol $C_1'$ proton peak at 4.92 ppm is coupled to proton peaks at 4.52 ppm and 4.36 ppm. From the magnitude of the coupling constant (14.2 Hz), the peak at 4.52 ppm can be assigned to the second ribitol $C_1'$ proton; and, by elimination, the peak at 4.36 ppm can be assigned to the $C_2'$ proton. The remaining ribitol protons can be assigned by following the connectivities shown in Figure 2. Homonuclear triple resonance experiments carried out on the ribitol $C_5'$ protons and $C_4'$ proton indicate that the $C_5'$ proton resonance at 4.18 ppm is coupled to an FAD phosphorus atom with a coupling constant of 7.6 Hz. In previous work (3) the peaks at 4.18 ppm and 4.08 ppm were assigned to the $C_4'$ and $C_2'$ protons, respectively, and the peak at 4.36 ppm was not resolved. It is interesting that distinct chemical shifts are observed for each proton on the ribitol $C_1'$ and $C_5'$ carbons. This and the magnitude of the ribitol proton-proton coupling constants indicate that even at 70 °C FAD has a stable preferred conformation. It would be interesting to study the effects of FAD concentration and temperature on the magnitude of the $C_1'$ and $C_5'$ proton coupling constants.

## References

1.) J. Jeener, Ampère International Summer School, Basko Polje, Yugoslavia, 1971.

2.) Bax, A. and Freeman, R. J. Mag. Res. 44, 542-561 (1981).

3.) Kainosho, M. and Kyogoku, Y., Biochemistry 11, 741 (1972).

(Received in USA 4 October 1982)