Photo-C.I.D.N.P. in Nucleic Acid Bases and Nucleotides

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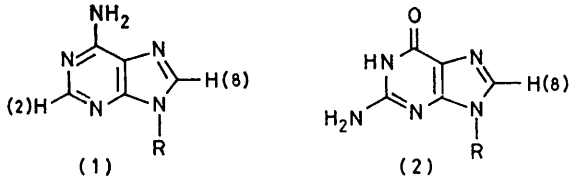
Summary Photo-C.I.D.N.P. (chemically induced dynamic nuclear polarization) is generated in nucleic acid bases (purines and pyrimidines) and in purine nucleotides in a cyclic reaction with a photo-excited flavin.

FLAVIN-SENSITIZED photo-C.I.D.N.P. (chemically induced dynamic nuclear polarization) has previously been observed in the 360 MHz n.m.r. spectra of the amino-acids tyrosine, histidine, and tryptophan.[1,2] This effect can be used to

obtain information about the surface structure of globular proteins and their interactions.[2,3] We report here that nuclear spin polarization can be generated similarly in nucleic acid bases and nucleotides.



^{1}H Photo-C.I.D.N.P. spectra were taken by laser irradiation in the probe of the Bruker HX-360 n.m.r. spectrometer operating in the pulse Fourier transform mode.[1] Difference spectra were obtained by taking alternating 'light' and 'dark' free induction decays and subtracting the resulting spectra. For the light spectrum the sample containing a D_2O solution of the substrate and 3-carboxymethyllumiflavin was irradiated with 0·6 s pulses from an argon ion laser (6 W, multiline mode, light power mainly at 488 nm and 514 nm). Figure 1 shows the result for adenosine-5′-monophosphate (**1**, R = ribose-5-phosphate; AMP).

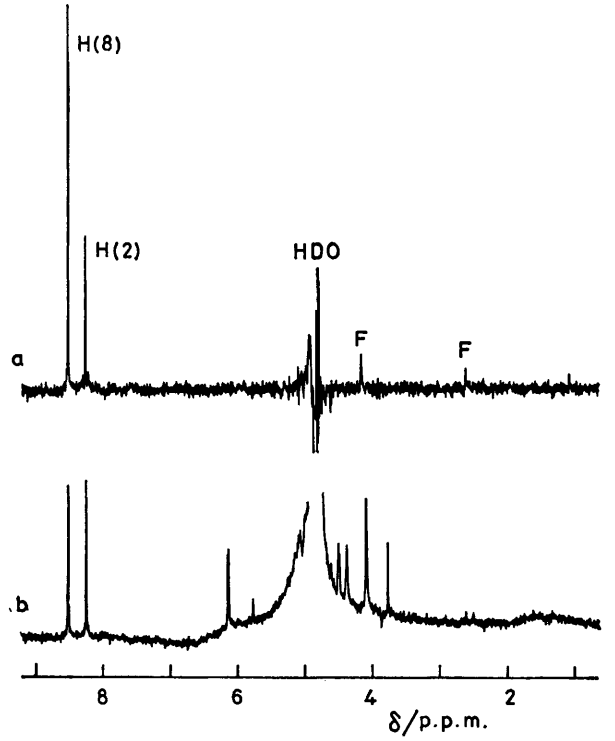


FIGURE 1. (a) 360 MHz photo-C.I.D.N.P. difference spectrum (light *minus* dark) of 5 mM AMP and 0·4 mM flavin in D_2O, pH 6·2, 22 °C, 10 scans; (b) dark spectrum. Lines indicated by F belong to the flavin.

The difference spectrum (Figure 1a) shows positive enhancements for H(8) and H(2) of the adenine ring at δ 8·52 and 8·25 respectively. Small positive effects at δ 2·52 and 4·15 belong to methyl groups of the flavin.[1]

A similar result is obtained for guanosine-5′-monophosphate (**2**, R = ribose-5-phosphate; GMP) as is shown in Figure 2. Enhanced absorption is observed for the H(8) ring proton.

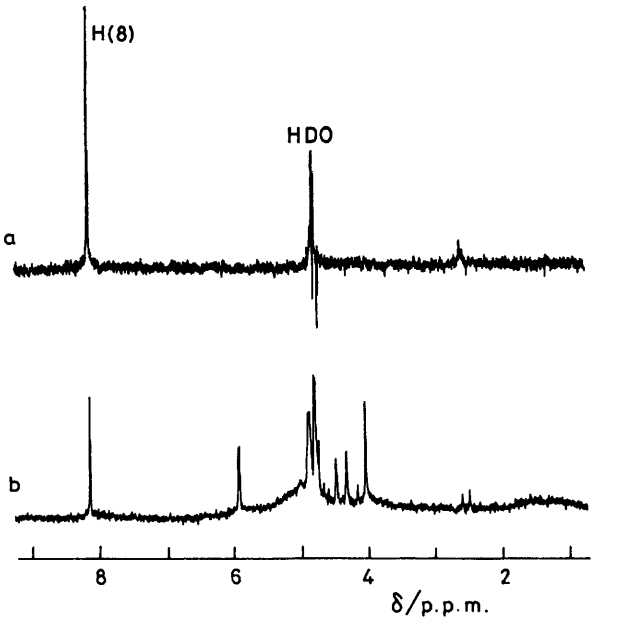


FIGURE 2. (a) 360 MHz photo-C.I.D.N.P. difference spectrum of 4 mM GMP and 0·4 mM flavin in D_2O, pH 6·5, 22 °C, 10 scans; (b) dark spectrum.

The reaction mechanism is likely to involve electron transfer from the nucleic acid bases (N) to the triplet flavin (^{3}F) as indicated in reactions (1) and (2).[4] Nuclear

$$F \xrightarrow{h\nu} {}^1F \rightarrow {}^3F \quad .. \quad .. \quad .. \quad (1)$$

$$^3F + N \rightarrow F^{\bullet -} + N^{\bullet +} \quad .. \quad .. \quad (2)$$

$$F^{\bullet -} + N^{\bullet +} \rightarrow F + N \quad .. \quad .. \quad (3)$$

spin polarization is then generated in the back electron transfer step (3). On the basis of reactions (1)—(3) the sign of the polarization[5] is in accordance with negative hyperfine coupling constants for H(8) and H(2) in the AMP derived radical [and for H(8) in the GMP radical], and with *g*-factors of these radicals lower than that of the flavin semiquinone. The electron transfer reaction is highly reversible since there is no indication of polarized products other than the reactants. Similar C.I.D.N.P. effects have been observed for the free purine bases adenine (**1**, R = H) and guanine (**2**, R = H) and for adenine-containing coenzymes such as NAD(P), ADP, ATP, and for FAD, which has a built-in flavin unit. However, the situation is different for the pyrimidines. Whereas strongly enhanced absorption was observed for the methyl group of thymine, no polarization could be detected in the case of thymidine. This suggests that for thymine the primary step in the reaction with triplet flavin is abstraction of the hydrogen atom at the N(1) position, which is no longer available in the nucleoside. Similarly, C.I.D.N.P. could be observed in the methylated pyrimidines, 3-methylcytosine and 5-methylcytosine, but not in cytosine itself, in uracil, or in the corresponding pyrimidine nucleosides.

The present results bear on the mechanism of dye-sensitized photo-oxidation of nucleic acids (photodynamic action).[6] Of the two basic mechanisms of this effect,

type I (radical), and type II (singlet oxygen), reactions (1)—(3) represent the first steps of the type I mechanism, which therefore can be studied by the photo-C.I.D.N.P. method. We are currently investigating the nucleotide polarizations under various conditions of temperature, pH, and ionic strength, and extending the measurements to oligonucleotides in order to ascertain the effects of base pairing and base stacking. The photo-C.I.D.N.P. method could be very useful as a probe for tertiary structure elements of naturally occuring polynucleotides and it may allow the study of protein–nucleic acid interactions not only from the protein side,[3b] but from the nucleic acid side as well.

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