Photo-CIDNP in the Tyrosyl Unit: A New Tool in High Resolution Nuclear Magnetic Resonance Studies of Peptides

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Summary Photo-CIDNP in the ring and in the methylene protons of the tyrosyl unit in tyrosine, in its derivatives, and in peptides dissolved in D_2O is induced by the reversible hydrogen abstraction by excited triplet xanthene dyes.

WE report a novel application of photochemically produced CIDNP to high resolution n.m.r. spectroscopy of the tyrosyl unit in polypeptides. Previous studies[1–4] have shown that strong ^1H (and ^{13}C[5]) CIDNP is observed in a variety of phenols (ArOH) during reversible hydrogen atom transfer to several acceptor molecules (S) (such as xanthene dyes,[1,2] triphenylmethane dyes,[2] and nitro-aromatic compounds[2–4]) light-excited to their triplet states. In these systems two H-transfer processes take place which are responsible for the observed polarization as shown in equations (1) and (2).

Hydrogen abstraction:

$$\text{ArOH} + {}^3\text{S} \rightarrow \text{ArO}^\cdot + \text{SH}^\cdot \qquad (1)$$

Recombination:

$$\text{ArO}^\cdot + \text{SH}^\cdot \rightarrow \text{ArOH} + \text{S} \qquad (2)$$

In the systems examined thus far processes (1) and (2) are highly reversible in the chemical sense, no stable products being obtained when the light is shut off.

Extending these studies to the tyrosyl unit, we were able to observe strong nuclear polarization in the tyrosyl ring and methylene protons when D_2O solutions of compounds containing the tyrosyl unit are u.v.-irradiated in the presence of 1—5 × 10^{-4} M fluorescein or of its polyhalogenated derivatives, Rose Bengal, Erythrosin B, and Eosin Y.†

Apart from tyrosine (as the ^2H hydrochloride) and its *N*-acetyl and *N*-trifluoroacetyl derivatives, strong CIDNP was also observed from several peptides including tyrosylglycine (p-$HOC_6H_4CH_2CHNH_2CONHCH_2CO_2H$)and tryptophanyltyrosine. The Figure shows typical spectra obtained for a D_2O solution of tyrosylglycine. In this system the tyrosyl methylene protons show a *ca.* seven-fold enhancement (*A*) effect and the two ring protons *ortho* to the tyrosyl OH group show a similar emission effect (*E*). Very similar results are obtained for other systems. CIDNP is observable only in neutral or acid solutions.

The present results were all obtained using the previously described optical irradiation set-up.[2] Optical irradiation preceding the r.f. pulse, in the FT mode, as recently suggested by Closs,[6] gave similar results to CW or FT measurements using continuous light. Under the conditions of this work, no CIDNP could be observed in other aromatic amino-acids such as tryptophan, histidine, and phenylalanine.

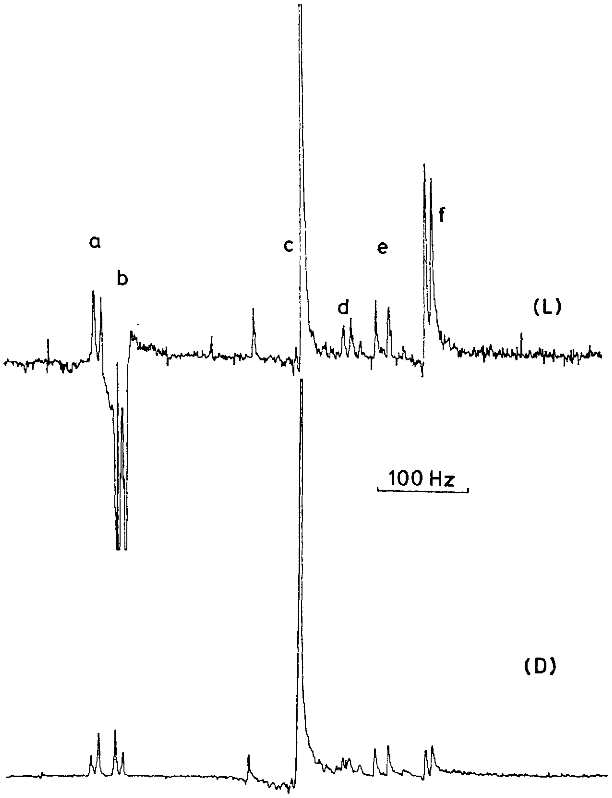

FIGURE. ^1H (90 MHz) FT n.m.r. spectra of tyrosylglycine (0·1 M) in D_2O, in the presence of 10^{-4} M fluorescein: (D), measured in the dark; (L), followeng light pulse. Amplitude in (L) is approximately twice that in (D). a, b, d, and f represent tyrosyl protons: a, ring protons *meta* to hydroxy group; b, ring protons *ortho* to hydroxy group; d, methine proton; f, methylene protons. c, HDO present in solvent; e, glycine methylene protons.

The previous analysis[1–4] of the signs of the observed polarizations based on the Kaptein rules[7] is entirely applicable to the tyrosyl ^1H CIDNP. The polarization arises probably both from recombination in the triplet geminate pair $^3\overline{\text{ArO}^\cdot + \text{SH}^\cdot}$ and in the encounter pair $^F\overline{\text{ArO}^\cdot + \text{SH}^\cdot}$ though the relative importance of the two to the observed polarization remains to be determined.

Very recently, much higher enhancements were obtained by using lower substrate concentrations than those used to obtain spectra in the Figure. Thus a maximum *E* effect of *ca.* 80 is obtained for the *ortho* ring protons at 0·02 M concentration of the tyrosine compound.

Other tyrosine peptides of greater complexity are being currently examined in order to establish the scope of this

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† We have been informed by Dr. R. Kaptein (Groningen) that similar studies are being carried out by him with other unspecified dyes.

method and the role of different factors such as electron exchange,[4] higher order structures, size, and tyrosyl unit accessibility.

Tyrosine CIDNP due to H atom abstraction by acetophenone has been reported by Lawler *et. al.*[8] The preliminary results in this investigation were obtained in collaboration with Dr. M. Weinstein.

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