Biochemical Applications of Chemically Induced Nuclear Polarization in Phenols, Peptides, Catecholamines and Related Molecules

By K. A. MUSZKAT,* M. WEINSTEIN* and C. GILON†

*Department of Structural Chemistry, The Weizmann Institute of Science, Rehovot, Israel, and

†Department of Organic Chemistry, The Hebrew University, Jerusalem, Israel

(Received 18 May 1978)

A photochemical method for producing proton nuclear polarization in phenolic biogenic molecules such as tyrosyl polypeptides and phenolic amines is described. The nuclear polarization is due to the reversible hydrogen-atom transfer from the phenolic hydroxy group to triplet photoexcited xanthene-dye molecules. Typical results obtained with insulin, catecholamines and other phenolic molecules are treated in detail.

Chemically induced nuclear magnetic polarization ('CIDNP'; hereafter called simply 'polarization') has been recently applied in studies of the hydrogen-atom transfer between phenols and H-atom-abstracting molecules photoexcited to their triplet state (Muszkat & Weinstein, 1975, 1976; Muszkat, 1977a).

These investigations have shown that the hydrogen atom is transferred from the OH-group of the phenol (ArOH) to the photoabstractor molecule (S), excited to its triplet state, and back, as described in steps (1)–(3):

The hydrogen-atom transfer in steps (2) and (3) is a virtual reaction as it results in no net chemical change. In the systems examined thus far the hydrogen-photoabstractor molecules were xanthene dyes, such as Fluorescein and its polyhalogenated derivatives, triphenylmethane dyes, nitroaromatic compounds and various oxo compounds. The hydrogen-transfer process is very efficient and highly reversible chemically, i.e. no stable chemical species being formed in the majority of cases studied when the optical irradiation is stopped.

Processes (1)–(3) when carried out in the presence of an external magnetic field, in the sample cell of an n.m.r. spectrometer, results in very significant positive or negative proton signal enhancements. The most pronounced effects are observed in the phenol protons (Muszkat & Weinstein, 1975, 1976a), and in some cases polarization is observable also in the dye nuclei (Muszkat & Weinstein, 1976a; Muszkat, 1977a). Maximum apparent positive enhancements of about 400 were obtained in the 4-methyl protons of 4-methyl-2,6-di-*t*-butylphenol (Muszkat & Weinstein, 1975, 1976a). Such effects clearly offer

numerous analytical, structural and mechanistic possibilities for n.m.r. studies of systems of biochemical interest that abound in phenolic structures of one or another type. In the initial study in this direction (Muszkat & Weinstein, 1976a), strong enhancement was observed in the signals of the ring-methyl protons of α -tocopherol during optical irradiation in the presence of xanthene dyes such as Fluorescein and Rose Bengal. More recently we reported strong polarization effects in such photoabstraction processes involving tyrosyl peptide segments acting as phenolic partners (Muszkat, 1977; Muszkat & Gilon, 1977, 1978). The systems investigated in this context include simple tyrosine derivatives, dipeptides (H-Tyr-Gly-OH, H-Tyr-Tyr-OH and H-Trp-Tyr-OH), the tripeptide H-Tyr-Tyr-Tyr-OH, the pentapeptide [leucine]enkephalin and the decapeptide luliberin and some of its derivatives. Under the conditions of these studies (see below), polarization is specific to the α -methylene and ring protons of the tyrosyl unit.

In the present paper we describe the extension of the phenol polarization studies to two different directions.

(a) Polypeptides

Whereas previous work has shown that this method may be applied to ten-member peptides, the present results clearly indicate that even with a 90 MHz spectrometer it is possible to obtain clear-cut tyrosyl polarization in peptides of much larger size, the case studied in detail being that of bovine insulin, a molecule containing 51 amino acid residues of which four are tyrosine. This result enlarges considerably the scope of polarization investigations in this field, suggesting new possibilities that seem accessible with superconducting magnet spectrometers. These studies have to await the development of suitable optical irradiation systems for use with such spectrometers.

(b) Catecholamines

Polarization due to hydrogen photoabstraction has been observed in a variety of catecholamines and of related molecules, thus illustrating the generality of the dye method for obtaining nuclear polarization and suggesting also new ways of studying some of the processes in which these molecules take part.

Methods and Materials

The solvent in these studies was $^2\text{H}_2\text{O}$ (Aldrich, 100%) or its mixture with $\text{C}^2\text{H}_3\text{CN}$ or $\text{C}^2\text{H}_3\text{O}^2\text{H}$. The photoabstractors mostly used were Fluorescein, Rose Bengal, Erythrosin B and Eosin Y. Other photoabstractors such as $[\text{}^2\text{H}_6]\text{acetone}$, anthraquinone-2-sulphonic acid and aromatic ketones might also be effective, on the basis of limited experience available from the previous studies. The samples of insulin (bovine ZnCl_2 -modified insulin, obtained from Sigma, St. Louis, MO, U.S.A.; catalogue no. I 5500, approx. 24 i.u. per mg, about 0.5% Zn) were repeatedly freeze-dried from excess 100% $^2\text{H}_2\text{O}$ containing dilute ^2HCl . This exchange of labile protons was also carried out with the other peptides in this series, its purpose being mainly to decrease the intensity of the H^2HO signal. Noradrenaline, tyramine and 3-hydroxytyramine, as the hydrochlorides, and adrenaline were obtained from Sigma. The sample of L-dopa (3,4-dihydroxyphenylalanine) was obtained from Fluka, Buchs, Switzerland.

The ^1H n.m.r. measurements were performed on a Bruker HFX-10 spectrometer operating at 90 MHz. The optical irradiation was carried out with a 2500 W mercury/xenon lamp, the light being led horizontally to the sample cell by means of a 50-cm-long light-guide. This optical-irradiation set-up has been described in detail elsewhere (Muszkat & Gilon, 1977). Intermittent (light-pulse/radio-frequency-pulse measurement) and continuous optical excitations were used, both yielding usually similar results. For a discussion and comparison of optical-excitation methods in chemically induced nuclear magnetic polarization, the recent report of Schaublin *et al.* (1977) should be consulted. The measurements were carried out in most cases in the Fourier-transform mode, though continuous wave measurements gave usually similar results whenever the comparison was tried out. The pD values are corrected for the isotope effect (cf. Glasoe & Long, 1960).

Results and Discussion

Insulin

Fig. 1 shows the effect of optical excitation on the 90 MHz proton n.m.r. spectrum of a 9.7 mM solution of bovine insulin at pD 2.4 in the presence of Fluores-

cein (1.3 mM) acting as hydrogen-atom photoabstractor. Signal A, the highest-field aromatic-proton signal in spectrum (b), is due to the two tyrosyl aromatic ring protons *ortho* to the hydroxyl group. Optical excitation (spectrum a) produces a net emission effect, as required by this assignment. Lower values of the Fluorescein/insulin ratio result in smaller extents of polarization. The results at pD 2.0 are entirely similar, but at pD 2.9 the polarization is significantly lower. More recently we have observed similar tyrosine polarization effects in bovine pancreatic trypsin inhibitor, with either Fluorescein or Fuchsin as hydrogen photoabstractors.

Results obtained with Fluorescein as H-atom photoabstractor

Noradrenaline. The effect of optical irradiation at pD 2.7 is shown in Fig. 2(a). In this case all aromatic

Fig. 1. Proton 90 MHz n.m.r. spectra of bovine insulin (21.5 mg in 0.4 ml of $^2\text{H}_2\text{O}$, 9.7 mM) in the presence of 1.3 mM-Fluorescein

(a) 1 s optical irradiation preceding each radio-frequency pulse; (b) dark spectrum. Each spectrum is the result of 128 accumulated radio-frequency pulses. A, Tyrosyl ring protons, *ortho* to OH-group; B and B', H^2HO impurity signals.

Fig. 2. Proton 90 MHz n.m.r. spectra of biogenic phenol amines in the presence of 0.7 mM-Fluorescein in $^2\text{H}_2\text{O}$. Lower tracings show dark spectra; upper tracings show spectra immediately after optical excitation. In (a)–(d) only ring proton signals are shown. Each spectrum results from 64 accumulated radio-frequency pulses. (a) Noradrenaline, 23 mM (1.3 mM in Fluorescein); (b) L-dopa, 32 mM; (c) 3-hydroxytyramine, 16 mM; (d) and (e) tyramine, 18 mM. In (d): A, ring protons *meta* to OH; B, ring protons *ortho* to OH. In (e): A, β -methylene protons; B, α -methylene protons.

protons appear in emission. For a 23 mM concentration of the amine, the enhanced-emission effect of about -7 seen in Fig. 2(a), is largest at a 1.3 mM concentration of the dye. A much smaller effect was observed at a 5-fold lower concentration of the dye. Similar nuclear-polarization effects were observed for adrenaline.

L-Dopa. Similar polarization effects were obtained at pD 4.7 (Fig. 2b) and at pD 3.1, and a much smaller effect was observed at pD 8.1. Again, in this case, all aromatic protons appear in emission. A weak enhancement was observed in the α -methylene protons.

3-Hydroxytyramine. At pD 7.4 the nuclear polarization is very weak. At pD 2.0 a strong effect is obtained. The ring protons (Fig. 2c) appear in emission as a multiplet, very much as in the case of L-dopa. The α -methylene protons show a weak enhanced-absorption effect.

Tyramine. At pD 2.0 a strong apparent polarization (-8 -fold) of the ring protons *ortho* to the OH-group and a $+6$ -fold effect on the α -methylene protons are obtained. The magnitude of the effect and the aromatic-protons pattern resemble closely the results obtained with di- and tri-peptides (Muszkát & Gilon, 1977). At pD 7.2 the polarization is considerably weakened.

Role of hydrogen-ion concentration

The present results clearly indicate that hydrogen-ion concentration is of decisive importance in determining the extent of polarization. Further insight into this effect is provided by the following results obtained with 10 mM-L-tyrosyl-L-tyrosine (Muszkát & Gilon, 1977) in the presence of 0.5 mM-Fluorescein. The enhancement of the signal of the ring protons *ortho* to the OH-group (light signal/dark signal) was 1 at pD 9.6 (e.g. no effect), -1 at pD 8.9, -3 at pD 6.6 and -5 at pD 2.0. Such effects seem to be due to several factors, two of which, dye binding by the substrate and electron exchange in polarized molecules, are most readily recognizable. Dye binding and immobilization by the substrate is suggested by the effect in the case of insulin caused by a change in the pD from 2.9 to 2.4–2.0. The other factor, the loss of nuclear polarization through exchange of a polarized tyrosyl unit that underwent ionization of the hydroxyl group (Ar^*O^-) with phenoxy radicals $\text{ArO}\cdot$, $\text{Ar}^*\text{O}^- + \text{ArO}\cdot \rightarrow \text{Ar}^*\text{O}\cdot + \text{ArO}^-$ (*indicates nuclear polarization), is strongly suggested by our work on nitroaromatic compounds (Muszkát, 1977a). This effect is expected to be of importance at pD values close to the pK of the phenolic compound (e.g. pD 10–8 in the case of tyrosyltyrosine).

The previous publications (e.g. Muszkat & Weinstein, 1976a) should be consulted for the analysis of the signs of the polarization effects.

References

- Glasoe, P. K. & Long, F. A. (1960) *J. Phys. Chem.* **64**, 188–190
- Muszkat, K. A. (1977a) *Chem. Phys. Lett.* **49**, 583–587
- Muszkat, K. A. (1977b) *J. Chem. Soc. Chem. Commun.* 872–873
- Muszkat K. A. & Gilon, C. (1977) *Biochem. Biophys. Res. Commun.* **79**, 1059–1064
- Muszkat, K. A. & Gilon, C. (1978) *Nature (London)* **271**, 685–686
- Muszkat, K. A. & Weinstein, M. (1975) *J. Chem. Soc. Chem. Commun.* 143–144
- Muszkat, K. A. & Weinstein, M. (1976a) *Z. Phys. Chem. Frankfurt am Main* **101**, 105–114
- Muszkat, K. A. & Weinstein, M. (1976b) *J. Chem. Soc. Perkin Trans. II* 1072–1074
- Schaublin, S., Wokaun, A. & Ernst, R. R. (1977) *J. Magn. Reson.* **27**, 273–302