A photo-CIDNP investigation of tyrosine mobility and exposure in human β-endorphin in the presence of phospholipid micelles

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1. INTRODUCTION

β-Endorphin, the C-fragment of lipotropin isolated from pituitary glands [1–3] and brain [4,5], has potent opiate-like properties [2,6]. The neuropeptide consists of a single chain of 31 amino acid residues, the integrity of which is necessary to maintain full biological activity [7]. The 1–5 N-terminal peptide forms the complete sequence of Met-enkephalin and plays a crucial role in the function of all endorphins [8].

Tyr-Gly-Gly-Phe-Met^{5}-Thr-Ser-Glu-Lys-Ser^{10}-Gln-

Thr-Pro-Leu-Val^{15}-Thr-Leu-Phe-Lys-Asn^{20}-Ala-Ile-

Ile-Lys-Asn^{25}-Ala-Tyr-Lys-Lys-Gly^{30}-Glu

CD studies indicate that the molecule adopts a predominantly random conformation in aqueous solution which changes to a highly α-helical structure on interaction with anionic surfactants or lipid micelles [9,10]. This secondary structure has been postulated to be important in binding to the receptor and thus for biological activity [10,11]. Such non-specific binding may facilitate the search for the receptor site by reducing the dimensionality of the diffusion process [12].

Abbreviations: CIDNP, chemically induced dynamic nuclear polarization; NMR, nuclear magnetic resonance; $C_{12}PN$, *n*-dodecylphosphorylcholine; CD, circular dichroism

In human β-endorphin Tyr-1 and Tyr-27 appear to be natural probes for the study of peptide-lipid interactions. The former, located in the N-terminal fragment, is probably primarily involved in the binding to the receptor; the latter lies in a region characterized by high helix propagation probability [13] containing cationic lysyl side chains which are reported to bind to anionic lipid head groups [9,14].

In this paper we report a photo-CIDNP investigation into the binding of *n*-dodecylphosphorylcholine micelles to human β-endorphin. The results indicate that the accessibility *and* mobility of both tyrosines are reduced in the presence of the lipid, both effects being more pronounced for Tyr-27 than for Tyr-1. These observations are analysed semi-quantitatively by means of a simple model of cross polarization [15].

2. EXPERIMENTAL

Human β-endorphin was obtained from Bachem Feinchemikalien A.G. and used without further purification. 3-*N*-carboxymethyllumiflavin and *n*-dodecylphosphorylcholine ($C_{12}PN$) were the generous gifts of Dr F. Müller (Wageningen) and Dr M.R. Egmond (Utrecht) respectively. D_2O (99.95%) was from Merck Isotopes.

Photo-CIDNP spectra [16,17] were recorded at 360 MHz on a Bruker HX-360 spectrometer equipped with an Aspect 2000 computer. Chemical shifts are quoted relative to sodium 3-trimethylsilyl [2,2,3,3-$^{2}H_4$]propionate (TSP).

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3. RESULTS

Figures 1A and 1B show respectively the aromatic regions of 270 MHz ^{1}H-NMR and 360 MHz photo-CIDNP spectra of human β-endorphin at pH 6.3. Both tyrosine residues are strongly enhanced exhibiting characteristic polarization patterns [16] (ε protons, emissive and δ protons, absorptive). The indicated assignments are the result of combined nuclear Overhauser and spin decoupling experiments [18,19]. Figure 1B shows that the two tyrosines in the native protein are completely accessible to the flavin dye used to generate spin polarization.

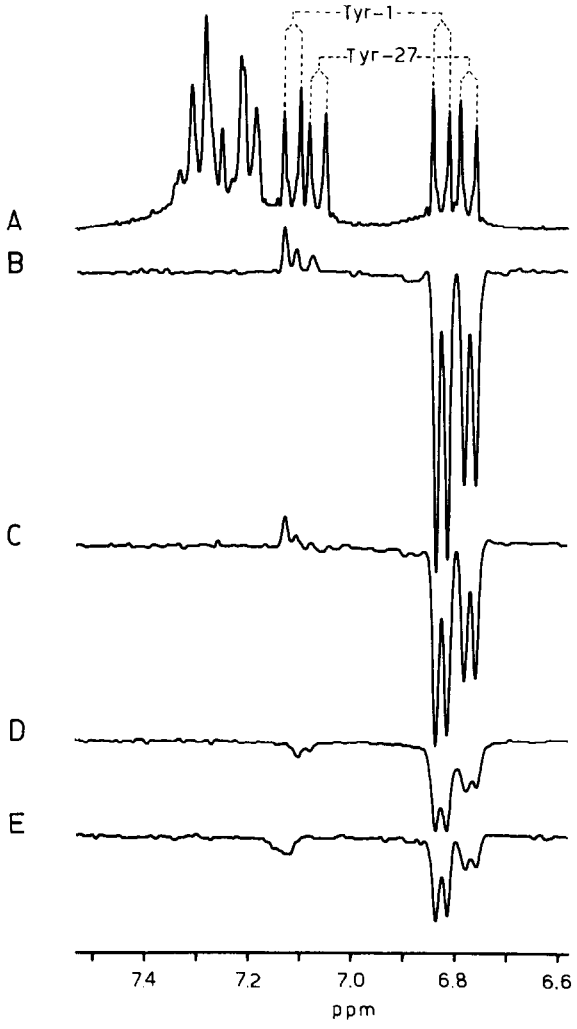

Fig.1. (A) 270 MHz ^{1}H-NMR spectrum of 0.5 mM human β-endorphin in D_2O. (B) 360 MHz ^{1}H photo-CIDNP difference spectrum of protein alone and in the presence of 2 mM (C), 7 mM (D) and 14 mM (E) *n*-dodecylphosphorylcholine micelles. pH 6.3, 0.5 mM flavin dye.

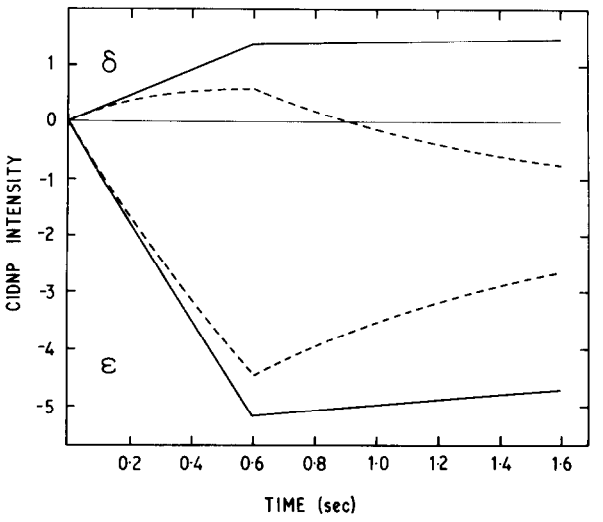

Fig.2. Calculated time dependence of 360 MHz CIDNP intensities (arbitrary units) for tyrosine δ and ε protons during ($0 < t < 0.6$ s) and after ($t > 0.6$ s) laser illumination. Solid curves, τ_c = 50 ps; broken curves, τ_c = 2 ns. See text and ref. [15] for details of calculation. Negative intensities correspond to emission, positive to absorption.

In the presence of 2 mM C_{12}PN lipid micelles (fig.1C) the Tyr-27 lines decrease relative to those of Tyr-1. On addition of more lipid (fig.1D and 1E) a further reduction of the Tyr-27 ε signal is observed while the δ resonance changes phase to emission. Similar, but less pronounced effects are found for Tyr-1: thus 14 mM lipid is required to invert the δ resonance compared to only 2 mM for Tyr-27. Moreover the Tyr-27 lines are broadened to a greater extent than those of Tyr-1.

These observations reflect a differential influence of lipid binding on the two Tyr residues in β-endorphin. Relative to Tyr-1, Tyr-27 becomes less exposed to the flavin dye, and its mobility more restricted, in the presence of micelles. The latter is evidenced not only by the increasing linewidths but also by the inversion of the δ-resonances.

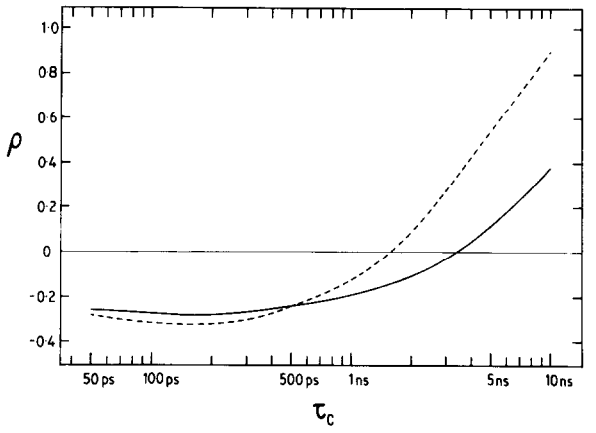

Fig.3. Calculated dependence of the 360 MHz CIDNP intensity ratio ρ ($\equiv I_\delta/I_\varepsilon$) on τ_c at 0.05 s (solid curve) and at 0.50 s (broken curve) after a 0.6 s light pulse.

Caused by cross relaxation from the ε protons, this effect becomes more important as the mobility of the tyrosine is reduced [15,20,21].

These effects can be analysed by means of a very simple model [15]. We assume that the motional modulation of the δ-ε proton dipolar interaction has an exponential autocorrelation function (correlation time, τ_c) and neglect all other sources of relaxation. Two sample calculations are shown in figure 2, for τ_c = 50 ps and 2 ns. During the light pulse ($0 < t < 0.6$ s) both δ and ε CIDNP signals grow with initial rates determined by the appropriate hyperfine couplings in the tyrosyl radical [22]. The effects of cross relaxation between the two nuclei can be seen clearly. From simulations of this type we determined the CIDNP intensity ratio, $\rho = I_\delta/I_\varepsilon$, as a function of τ_c at delays of 0.05 s and 0.5 s after a 0.6 s light pulse (fig.3). At τ_c = 500 ps the two quantum ($\alpha\alpha \leftrightarrow \beta\beta$) and zero quantum ($\alpha\beta \leftrightarrow \beta\alpha$) cross-relaxation rates (W_2 and W_0 respectively) are equal and no net cross polarization transfer occurs. At this value of τ_c, therefore, the two curves in fig.3 cross giving a δ/ε intensity ratio equal to the ratio of CIDNP production rates. At lower τ_c W_2 is greater than W_0, producing a small increase in $|\rho|$ while for $\tau_c > 500$ ps, the W_0 process dominates resulting in the inversion of the δ signal. These effects are closely similar to the dependence of the Nuclear Overhauser Effect on molecular mobility [23–25].

Table 1

Estimates of the motional correlation time, τ_c for tyrosine residues in human β-endorphin

Delay[a] (s)	$[C_{12}PN]$ (mM)	τ_c (ns)	
		Tyr-1	Tyr-27
0.5	0	1.0	1.3
0.5	2	1.1	1.6
0.5	7	1.4	2.2
0.5	14	2.2	5.2
0.05	0	1.6	2.0
0.05	14	2.6	3.4

[a] The delay after a 0.6 s light pulse

Using figure 3 one can obtain estimates of τ_c from experimental measurements of ρ, as set out in table 1. From these results we conclude that Tyr-27 is indeed less mobile than Tyr-1, even in the absence of lipid, and that it becomes considerably more restricted when micelles are present.

4. CONCLUSIONS

The present results show that photo-CIDNP of tyrosyl residues is a useful probe to monitor the changes that occur when β-endorphin binds to lipid micelles. The information from CIDNP is twofold: first, the suppression of the CIDNP effect of the ε-protons indicates that both tyrosine side chains are rendered less accessible in the micelle complex, the effect being more pronounced for Tyr-27. A similar suppression of tyrosine polarization has been observed for colipase–micelle complexes [26]. Secondly, the changes in the cross-polarization effect for the δ-protons indicate that the motional correlation times τ_c for the tyrosines become longer upon micelle binding. Again the immobilization of Tyr-27 is stronger than that of Tyr-1. It may be noted that for peptides with M_r-values of ca. 3000 the cross-polarization effect is particularly sensitive to molecular motion as it involves a change in sign (at 360 MHz) when $\tau_c = 5 \times 10^{-10}$ s.

The detailed interpretation of the present results is less straightforward. In principle both the change in exposure and the immobilization of the

tyrosine rings could be due to direct interaction with the micelle or to the generation of a folded structure upon micelle binding (or both). At present we cannot clearly distinguish between these possibilities, although some preliminary results with β-endorphin from camel, in which Tyr-27 is substituted by a histidine, indicate that the strong effects observed for Tyr-27 are at least in part due to folding of the peptide chain. In the camel species His-27 remains fully exposed in the presence of micelles, whereas the behaviour of Tyr-1 is very similar to that of the human peptide, indicating that the N-terminal region is mainly responsible for micelle binding. This is consistent with previous studies [27] which suggest that, in the presence of membrane-bound proteases from the brain, the N-terminal tyrosyl residue is protected against proteolytic attack.

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