Eur Biophys J (1985) 11:187–193

European Biophysics Journal

© Springer-Verlag 1985

Photo-CIDNP ^{1}H-NMR of proteins: Exposure, mobility and orientation of the tyrosyl side chains

A. De Marco[1], L. Zetta[1], and R. Kaptein[2]

[1] Istituto di Chimica delle Macromolecole del CNR, Via E. Bassini 15/A, I-20133 Milano, Italy
[2] Physical Chemistry Laboratory, University of Groningen, Nijenborgh 16, NL-9747 AG Groningen, The Netherlands

Received July 31, 1984/Accepted September 17, 1984

Abstract. The photo-CIDNP technique is capable of giving information, not only on the degree of exposure of aromatic residues in proteins and on the correlation time of aromatic side chains, but also on the fine structure of β-CH_2 multiplets, whose J-pattern directly reflects the side chain orientation about χ^1. Moreover, the resonance filtering achieved in the difference spectra allows selective measurement of line broadenings in binding processes, which are directly related to the association constants (K_a). In this communication, data are reported concerning the exposure, mobility and orientation of the tyrosyl side chains in bovine PSTI at different pH values. The immobilization of tyrosyl residues in Met-enkephalin, human, and camel β-endorphin in the presence of lipid micelles is also investigated; the extent of interaction of the three peptides with C_{12}PN is discussed. The binding efficiency is considerably lower for the shorter peptide; of the two endorphins, the camel hormone exhibits a higher affinity for the micelles than the human analog.

Key words: H-NMR, photo-CIDNP, PSTI Met-enkephalin, β-Endorphins

Introduction

The identification of individual multiplets in crowded regions of protein ^{1}H-NMR spectra is a difficult goal to achieve, even with the increased resolution attainable in recently proposed 2D experiments (Marion and Wüthrich 1983).

Among the various techniques proposed to solve this problem, the most useful have proved to be those which simplify the resonance pattern by means of the so called "difference spectroscopy", usually employing on- and off-resonance irradiation during acquisition (Gibbons et al. 1975a) or between scans (Gibbons et al. 1975b), making use of spin-decoupling and NOE effects. However, difference spectra resulting from selective decoupling are often difficult to interpret, and NOE difference spectra are usually too low in sensitivity to allow the application of the digital routines, which are mandatory, in order to resolve the fine structure of the resonance lines. Moreover, both types of experiments lack selectivity if the perturbing radio frequency field is set in a crowded region of the spectrum.

An approach which has the advantage of both high sensitivity and specificity for certain aromatic amino acid residues is the well known photochemically induced dynamic nuclear polarization technique (photo-CIDNP, Kaptein 1982). The method generates selective enhancements in the NMR intensities of the side chain protons of the reactive residues (tyrosine, histidine, and tryptophan), which can be readily observed in the so-called "light-minus-dark" presentation. This technique is of particular interest, since the relatively few aromatic aminoacids present in a protein are usually distributed throughout the sequence, and therefore, they are ideally suited for monitoring the various protein domains. The aromatic groups are of great biochemical interest, because of their hydrophobic interactions, acid-base chemistry, hydrogen bonding, and ability to intercalate between other planar groups. From the NMR stand-point, aromatic rings induce ring current shifts and NOE among themselves and on aliphatic groups. The resonance pattern of Tyr and Phe spin systems is related to the degree of freedom of the side chains; pK-shifts in the pH-titration of His and Tyr may also reflect a restricted microenvironment.

Until now, the photo-CIDNP technique has been mainly used to investigate the exposure and mobility

Abbreviations: PSTI, pancreatic secretory trypsin inhibitor; CIDNP, chemically induced dynamic nuclear polarization; C_{12}PN, n-dodecylphosphorylcholine; NOE, nuclear Overhauser enhancement

of aromatic side chains (Kaptein 1982). Recently, it has been shown that the correlation time of the tyrosyl rings can be measured from the ratio of δ and ε resonances (Hore et al. 1982; Zetta et al. 1982). In the present communication it is shown how this approach can be usefully employed to detect small variations of mobility resulting from incipient collapse of the tertiary structure near the aromatic residues. Moreover, the enhancement achieved via nuclear spin polarization makes it possible to identify and analyze the β-CH_2 resonances, which are barely detectable in the conventional ^{1}H-NMR spectrum because of extensive overlap with other aliphatic resonances. The vicinal $^3J_{\alpha\beta}$ couplings measured in the β-CH_2 multiplets in photo-CIDNP difference spectra provide direct information on the geometry of the aromatic side chain (χ^1 angle). Finally the β-CH_2 resonances appear to be suitable probes for the evaluation of line broadenings induced by binding processes.

Materials and methods

Bovine PSTI was obtained as described elsewhere (De Marco et al. 1982b). Met-enkephalin, human, and camel β-endorphin were obtained from Bachem Feinchemikalien, AG, and used without further purification. 3-N-Carboxymethyllumiflavin and n-dodecylphosphorylcholine ($C_{12}PN$) were generous gifts of Dr. F. Müller (Wageningen) and Dr. M. R.Egmond (Utrecht), respectively. ^2H_2O (99.95%) was from Merck Isotopes.

Photo-CIDNP spectra were recorded on a Bruker HX-360 spectrometer equipped with an Aspect 2000 computer.

Difference spectra were obtained by taking "light" and "dark" FID's and subtracting the spectra after Fourier transformation. A Spectra Physics model 171 argon ion laser was employed as the light source. A 0.6-s light pulse (4 W, multiline) was used, with a 0.05-s delay before the 90° rf pulse. Four scans were accumulated for each spectrum.

Quoted pH values are meter readings, not corrected for isotope effects.

The conventional NMR spectra were obtained with a Bruker HX-270 spectrometer, also controlled by an Aspect 2000 computer. Chemical shifts are given in ppm from sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$) propionate (TSP).

Spectra shown in Figs. 1 and 3 were resolution enhanced via the Gaussian convolution routine (Ferrige and Lindon 1978) and the sine-bell routine (De Marco and Wüthrich 1976), respectively.

Results and discussion

Bovine pancreatic secretory trypsin inhibitor (Kazal, PSTI) is a small, globular protein ($M_r \approx 6{,}000$) consisting of 56 aminoacid residues in a single polypeptide chain with three disulfide bridges. The inhibitor contains two tyrosines, one free and one buried, at chain positions 20 and 31, respectively (De Marco et al. 1982b); these positions are the same as those observed for the porcine homolog (De Marco et al. 1979, 1982a). Tyr-20 exhibits the typical AA'XX' doublet-of-doublets pattern (Fig. 1) for a fast flipping aromatic ring (no preferential χ^2) over a wide pH and temperature range, and titrates with a pK_a near 11, as expected for deprotonation of an exposed OH group. Tyr-31 exhibits a spectral pattern characteristic of hindered motion of the aromatic side chain; at pH 5.2 and 25° C the ring proton resonances consist of one relatively sharp doublet (AA', hatched resonances in Fig. 1), and one broad signal (BC, hatched resonances in Fig. 1), a situation often encountered in protein spectra (De Marco et al. 1979; Wagner et al. 1976). A progressive loosening of the microenvironment about the aromatic ring of Tyr-31 (faster flipping about χ^2, see sketches in Fig. 1) is observed with either increasing temperature or decreasing pH. In the basic pH range the OH cannot be deprotonated below (at least) pH 12.0. At pH 1.0 the resonances from Tyr-31 appear sharpened, overlapping into an A_4 singlet (Fig. 1), still far, however, from a pattern indicative of free exposure. The same phenomenon is observed by raising the temperature at a higher pH: at pH 2.0 the Tyr-31 ring resonances coalesce into a singlet at 65° C (Fig. 1).

Consistent with the above observations, in photo-CIDNP experiments performed at pH 5.2 and pH 1.0, Tyr-31 does not give any response, indicating that the aromatic side chain is not exposed at either pH, whereas Tyr-20 gives a strong enhancement (Fig. 2B and D), in agreement with the OH exposure observed in the pH titration. The small, negative signal near 7.3 ppm in Fig. 2B (vertically expanded in the insert), originates from the δ protons of Tyr-20. Since the δ-ε proton dipolar interaction is motionally modulated, the CIDNP intensity ratio, $\varrho = I_\delta/I_\varepsilon$, can be calculated as a function of the correlation time of the aromatic ring τ_c, at various delays after the light pulse (Hore et al. 1982). With this method, at pH 5.0, with a delay of 0.05 s, a value of $\varrho \approx 0.06$ can be estimated for Tyr-20, which corresponds to a τ_c of approximately 4.2 ns. At pH 1.0, the signal from the δ protons is not easily detected, partly because of the poorer signal-to-noise ratio in the more dilute sample (see caption to Fig. 2). However, a small, positive signal-denoted by the arrow- is found at the position of the δ protons from Tyr-20 in Fig. 2D.

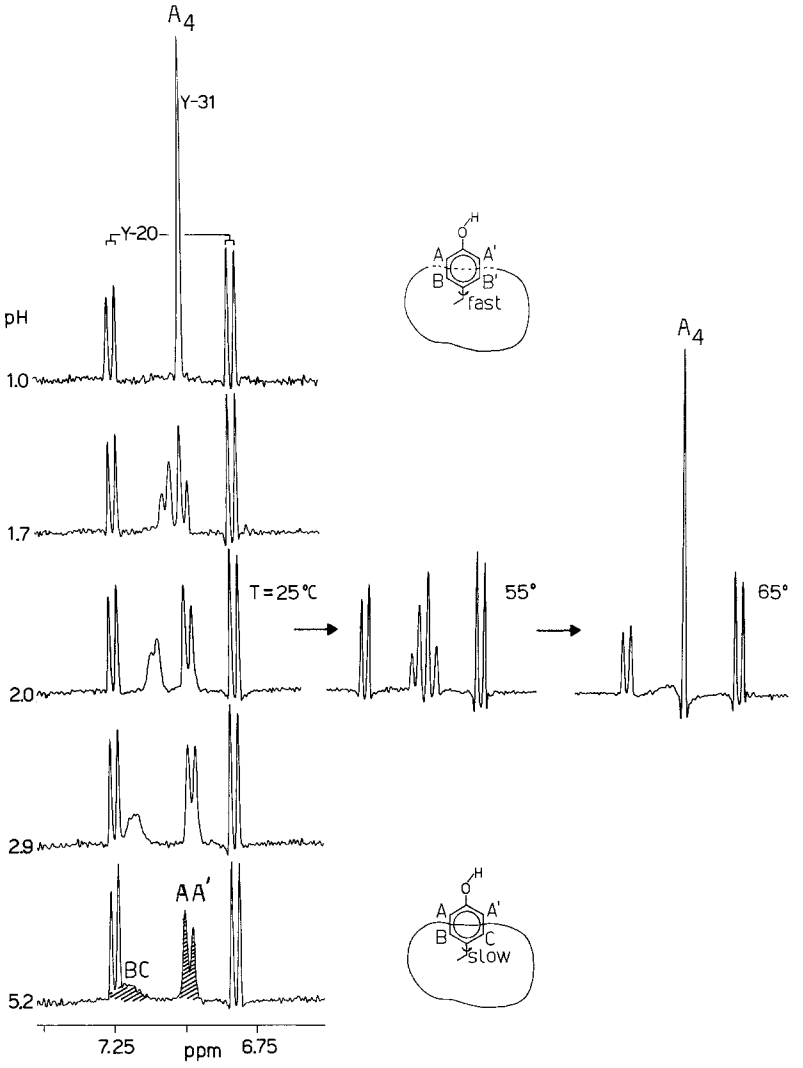


Fig. 1. ^{1}H-NMR of bovine PSTI: aromatic resonances. 270 MHz spectra at variable pH, as indicated on the left side, T = 25° C. At pH 2.0 two additional spectra are presented, recorded at 55° C and 65° C, the latter showing the coalescence of the Tyr-31 resonances into an A_4 singlet, similar to what is observed at pH 1.0, 25° C. The tyrosine sketches adjacent to the spectra at pH 5.2 and 1.0 show the transition from slow-to-fast flipping of the aromatic ring about the χ^2 angle. Solutions were ~ 2.5 mM in ^2H_2O. The resonances from Tyr-20 and Tyr-31 are marked in the spectrum at pH 1.0 (Y-20, Y-31). The spectral pattern of Tyr-31 is also indicated (AA'BC, pH 5.2; A_4, pH 1.0; A_4, pH 2.0, 65° C)

The partial loosening of the tertiary structure at pH 1.0 is expected to lower τ_c, and with the 0.05 s delay after the light pulse employed in the present experiments the ϱ vs τ_c curve crosses the $\varrho = 0$ axis for $\tau_c \approx 3.4$ ns (Zetta et al. 1982). At pH 1.0 a value for $\varrho \approx -0.04$ can be roughly estimated for Tyr-20, leading to a τ_c of ca. 2.9 ns.

This "inversion pattern", i.e., the change of sign of the tyrosyl δ resonances in the photo-CIDNP difference spectrum is caused by cross-relaxation from the ε protons, and reflects a change of mobility (Hore et al. 1982; Zetta et al. 1982). In human β-endorphin, at pH 6.3, the δ resonances from Tyr-27 are in absorption (positive); on progressive addition of C_{12}PN lipid micelles, they broaden and eventually change phase to emission (negative). This was interpreted as due to a change in exposure and immobilization of the aromatic ring, owing to the generation of a folded structure and (or) to direct interaction with the micelles (Zetta et al. 1982). By analogy, for bovine PSTI it can be concluded that on lowering the pH from 5.2 to 1.0, not only is the conformational pressure around Tyr-31 decreased, as manifested by the change in the spectral pattern (Figs. 1, 2A and C), but also the aromatic ring of Tyr-20, already free and exposed, gains further mobility. A strong photo-CIDNP effect (positive) is also observed on the β protons of Tyr-20, whose spin system, otherwise non-detectable because of the strong overlap among resonances near 3.0 ppm (Fig. 3A), is readily recovered in the enhanced photo-CIDNP difference spectrum at pH 5.2 (Fig. 3B). The doublet and triplet appearance of the β resonances suggests that one $^3J_{\alpha\beta}$ is small and one comparable to the geminal $^2J_{\beta\beta'}$. A computer simulation of the multiplets (Fig. 3C) gives 13.8 and 2.5 Hz for $^3J_{\alpha\beta}$ (the latter value is not observed as an actual splitting, but refined to best match the observed lineshapes). Since these values are essentially identical with the pure $^3J_{trans}$ and $^3J_{gauche}$ values

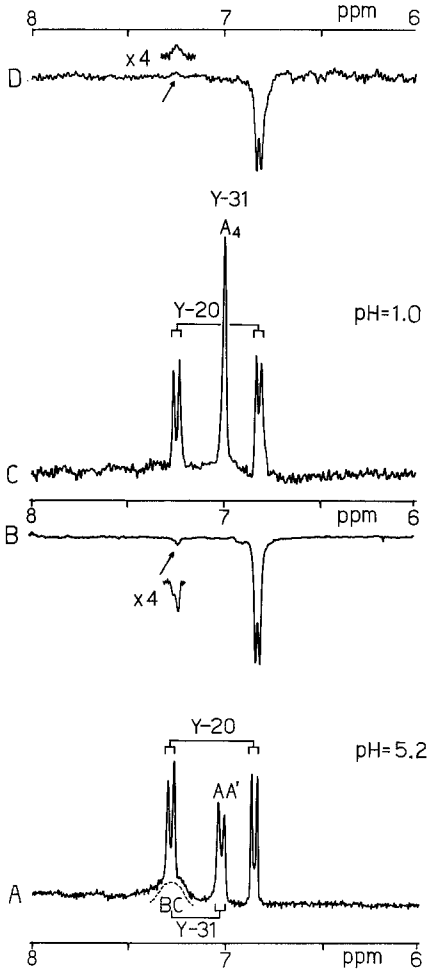


Fig. 2A–D. ^{1}H-NMR of bovine PSTI: aromatic resonances. (**A**): 270 MHz reference spectrum for experiment (**B**) solution, 2.5 mM in ^2H_2O, pH 5.0, 25° C; (**C**): 270 MHz reference spectrum for experiment (**D**), solution, ~ 1 mM in ^2H_2O, pH 1.0 25° C; (**B, D**): 360 MHz photo-CIDNP difference spectra at pH 5.0 and 1.0, respectively. The resonances from Tyr-20 and Tyr-31 and the spectral pattern of Tyr-31 are denoted as in Fig. 1. The arrows and the vertically expanded insets in spectra (**B**) and (**D**) refer to the δ resonances from Tyr-20 (see text)

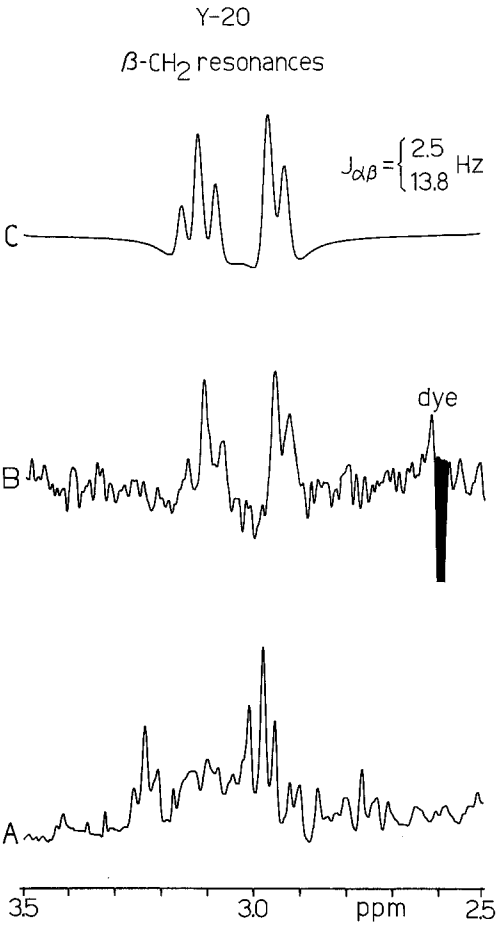
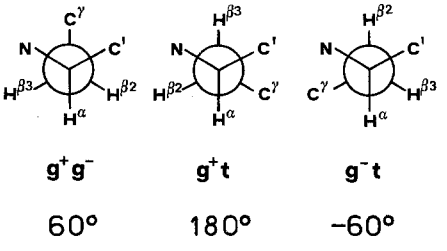


Fig. 3A–C. ^{1}H-NMR of bovine PSTI: spectral region between 2.5 and 3.5 ppm. Solution, 2.5 mM in ^2H_2O, pH 5.2, 25° C (**A**) reference spectrum at 270 MHz; (**B**) photo-CIDNP difference spectrum at 360 MHz; (**C**) computer simulation of (**B**). A negative signal from the flavin at 2.6 ppm has been blackened in spectrum (**B**)

proposed by Pachler (1964), i.e., 13.6 and 2.6 Hz, respectively, only one of the staggered rotamers about χ^1 can be essentially populated (the g^+t or the g^-t).



This conclusion implies that the motional freedom observed for the χ^2 orientations of the Tyr-20 sidechain does not extend to the next dihedral angle.

The X-ray structure of the complex between porcine PSTI and bovine trypsinogen has been refined to 1.8 Å resolution (Bolognesi et al. 1982). The amino acid conservancy between porcine and bovine inhibitors is extensive, involving 80% of the residues (De Marco et al. 1982b); in particular, both Tyr-20 and Tyr-31 are invariant and exhibit similar spectral features (De Marco et al. 1982a, b). From the crystallographic coordinates we calculated the dihedral angles (N-C^α-C^β-C^γ) = −178° and (C'-C^α-C^β-C^γ) = 64°. By looking at the above scheme, it is clear that these angles point to the g^+t isomer.

It appears resonable to conclude that no conformational changes involving the Tyr-20 orientation about χ^1 occur by dissolving PSTI in water.

The binding of Met-enkephalin and β-endorphins to phospholipid micelles has already been investigated by photo-CIDNP ^{1}H-NMR (Zetta et al. 1982, 1983). In those studies, attention was essentially focused on aromatic resonances, where variations of enhancement upon addition of $C_{12}PN$ (dodecylphosphorylcholine) were interpreted in terms of reduced mobility. A line broadening was also observed, although not strong enough to be quantitatively measured, especially in the case of the pentapeptide. In the photo-CIDNP difference spectra the β-CH_2 lines appear more suitable than the aromatic resonances for measuring linewidth variations, which potentially contain information on the binding parameters. Figure 4 shows the β-CH_2 resonances from Tyr-1 in Met-enkephalin (Tyr-Gly-Gly-Phe-Met) for the intact peptide (A), and in the presence of a 40 : 1 (B), and a 100 : 1 (C) molar excess of $C_{12}PN$. The multiplets have been computer simulated (A′−C′) to get an accurate estimate of the line broadenings in spectra B and C. The refined values are listed in Table 1.

The binding of human β-endorphin to $C_{12}PN$ micelles is considerably more efficient than that of the shorter peptide (its N-terminal fragment) under the same conditions. At much lower concentrations than those required to affect the β-CH_2 resonances from Tyr-1 in Met-enkephalin, the same signals in human β-endorphin are broadened to a much greater extent than in the pentapeptide. Figure 5 shows the β-CH_2 resonances from Tyr-1 and Tyr-27 in human β-endorphin at several $C_{12}PN$/peptide ratios. It has to be noted that the β-CH_2 tyrosyl resonances, already difficult to identify in the free peptide, could not be detected in the presence of the signals from the lipid protons in a conventional spectrum. Only the selec-

Table 1. Parameters associated with the interaction of Met-enkephalin, human, and camel β-endorphin with $C_{12}PN$ (n-dodecylphosphorylcholine) micelles

Met-enkephalin			Human β-endorphin			Camel β-endorphin		
$[L_0]$[a] (mM)	$\Delta\nu_E$[b] (Hz)	τ_c[c] (ns)	$[L_0]$ (mM)	$\Delta\nu_E$ (Hz)	τ_c (ns)	$[L_0]$ (mM)	$\Delta\nu_E$ (Hz)	τ_c (ns)
0		2.0	0		1.6	0		2.4
20	3.0	2.2	2	1.3	1.6	2	3.0	2.4
50	4.5	2.2	7	4.0	1.9	7	10.0	3.5
			14	10.0	2.6	14		3.6

[a] Total lipid concentration vs 0.5 mM peptide

[b] Line broadening caused on the Tyr-1 β-CH_2 resonances by the addition of lipid to the peptides, relative to the linewidth in the absence of $C_{12}PN$

[c] Correlation time of the Tyr-1 side chain (see text)

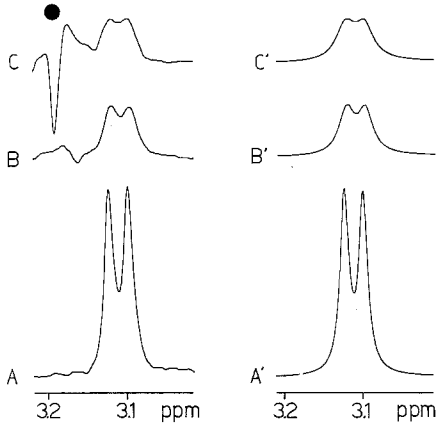


Fig. 4A−C. ^{1}H-NMR of Met-enkephalin: β-CH_2 resonances from Tyr-1. Solution, 0.5 mM in ^2H_2O, pH 6.3, 30° C. Photo-CIDNP difference spectra of the intact peptide (**A**), and in the presence of a 40 : 1 (**B**) and a 100 : 1 (**C**) excess of $C_{12}PN$ micelles. (**A′−C′**), computer simulation of (**A−C**), respectively. The black dot in (**C**) denotes incomplete cancellation of the $N(CH_3)_3$ lipid signal

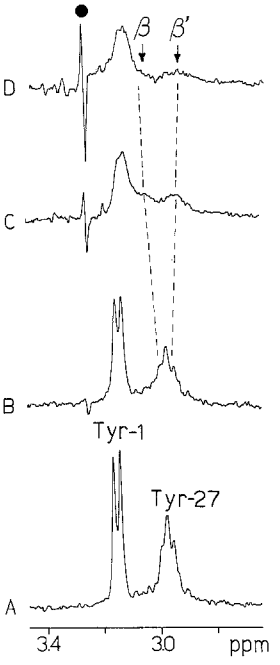


Fig. 5A−D. ^{1}H-NMR of human β-endorphin: β-CH_2 tyrosyl resonances. Solution, 0.5 mM in ^2H_2O, pH 6.3, 30° C. Photo-CIDNP difference spectra of the intact peptide (**A**), and in the presence of a 4 : 1 (**B**), 14 : 1 (**C**), and 28 : 1 (**D**) molar excess of $C_{12}PN$ micelles. The black dot in (**D**) denotes incomplete cancellation of the $N(CH_3)_3$ lipid signal. The doublet at 3.15 ppm results from the isochronous H^{β}'s of Tyr-1, and the multiplet near 3.0 ppm from those of Tyr-27. For the latter, the progressive increse of the relative chemical shift upon lipid addition is indicated by the dotted line connecting spectra **B, C,** and **D**

tive enhancement induced in the photochemical experiment makes possible selective detection of the multiplets in the difference spectra, devoid of all the undesired signals from both the peptide and the lipid. In human β-endorphin, as observed above for Met-enkephalin, the Tyr-1 β-CH_2 resonances do not exhibit any appreciable chemical shift contribution to the broadening upon binding. In fact, for Tyr-27 also, only the relative chemical shift between the β proton resonances varies (see dotted lines in Fig. 5), most likely reflecting a less averaged microenvironment around the two geminal protons, owing to the reduced mobility of the side chain in the presence of lipid micelles. For Tyr-1, in a region of the peptide which interacts less with the micelles, the β-CH_2 signals remain degenerate as a doublet all the way through the titration with C_{12}PN (Fig. 5A–D). By using the same spectral simulation approach as for Met-enkephalin, the line broadening values, listed in Table 1, were obtained. It is readily seen that the broadening is considerably larger for human β-endorphin than for its N-terminal pentapeptide, although the latter was exposed to much higher lipid concentrations. Camel β-endorphin differs from the human analog at sites 27 and 31, where Tyr and Glu are replaced by His and Gln, respectively. The analysis used for the Tyr-1 resonances in the human peptide is complicated, in the case of camel β-endorphin, by the interference between the β-CH_2 signals from His-27 and Tyr-1; the former, being negative, cause distortion and partial cancellation of the Tyr-1 multiplet. However a rough estimate was attempted, leading to the line broadening values also quoted in Table 1.

Assuming homogeneous binding, the Langmuir isotherm for complex formation can by expressed as

$$\frac{1}{f} = 1 + \frac{1}{[K_a\, L]}\,, \tag{1}$$

where K_a is the binding constant for association, $[L]$ is the molar concentration of free lipid and f is the fraction of bound peptide. To evaluate K_a, f has to be expressed in terms of NMR observables, most commonly the chemical shift, although, for example, the longitudinal ^{13}C relaxation time has been used to investigate the interaction of Met-enkephalin with phosphatidylserine vesicles in a biosynthetically enriched sample (Jarrel et al. 1980). Since in all three peptides the Tyr-1 β-CH_2 resonances do not exhibit appreciable chemical shift contributions to the broadening caused by interaction with the micelles, linewidths can be introduced in the Langmuir equation.

In conditions of fast exchange, it has been shown (Lanir and Navon 1971) that f is simply given by the ratio between the excess broadening $\Delta\nu_E$ and the linewidth of the bound peptide, i.e., the inverse of the transverse relaxation time of the bound peptide T_{2M}

$$f = T_{2M}\,\pi\Delta\nu_E\,. \tag{2}$$

For an excess of lipid concentration $[L] \approx [L_0]$ and (1) becomes

$$\frac{1}{\Delta\nu_E} = \pi\, T_{2M}\left[1 + \frac{1}{K_a[L_0]}\right], \tag{3}$$

Hence, the association constant K_a could in principle be evaluated from a plot of $1/\Delta\nu_E$ vs $1/[L_0]$. Figure 6A shows this plot for the three peptides, based on the values quoted in Table 1. Unfortunately, in order to obtain $1/K_a$, without knowing T_{2M}, both slope and intercept are required, and the latter is quite small. In other words, an accurate estimate of T_{2M} and, hence, K_a, required the use of a meaningless excess of lipid, or, conversely, peptide concentrations far below the limit of detection by the NMR method (Lanir and Navon 1971). Figure 6A indeed shows that the value of the intercept in the plot $1/\Delta\nu_E$ vs $1/[L_0]$ is below the experimental error.

It seems reasonable to expect essentially the same T_{2M} for complexes in which the peptide moieties differ only by two amino acid substitutions; with this assumption, the ratio between K_a for the human and for the camel hormone is directly given by the ratio between the corresponding slopes in Fig. 6A. It turns out that the camel peptide has a ca. three to four times higher affinity for the lipid micelles, than its human analog. Such increase of the binding strength is consistent with previous results obtained in the opiate receptor binding assay (Hammonds et al. 1982).

As mentioned above, when dealing with the trypsin inhibitors, the ratio (ϱ) between the intensities of δ and ε tyrosyl resonances in the photo-CIDNP difference spectra makes it possible, by means of a simple model, to calculate the correlation time of the aromatic side chain (Hore et al. 1982). Table 1 lists the τ_c's estimated for Tyr-1 in the three peptides at various lipid concentrations; two values have been published previously (Zetta et al. 1982). A τ_c vs $[L_0]$ plot is shown in Fig. 6B. The curves point to a definite correlation between the strength of binding and the immobilization of the peptide frame. The pentapeptide is very little affected in mobility by the addition of a large excess of lipid. Camel β-endorphin binds more tightly than the human analog, and its correlation time increases more quickly and reaches a

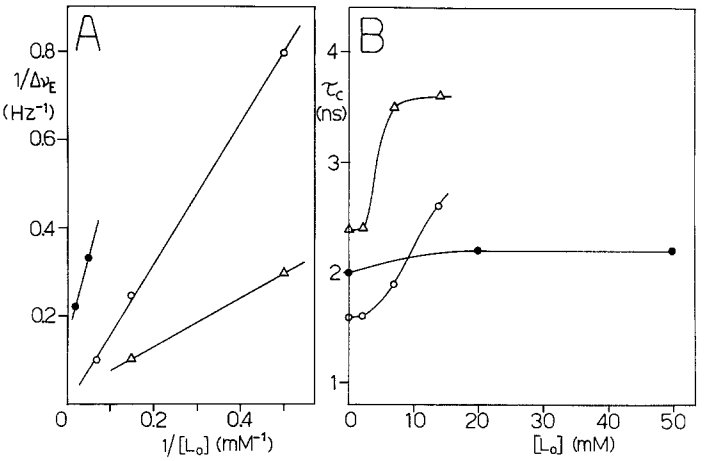


Fig. 6. A. $1/\Delta\nu_E$ vs. $1/[L_0]$ plot for Met-enkephalin (●), human (○), and camel β-endorphin (△). $\Delta\nu_E$ represents the net line broadening resulting from interaction with $C_{12}PN$, as measured on the Tyr-1 β-CH_2 resonances (see text); $[L_0]$ is the total lipid concentration. **B.** τ_c vs $[L_0]$ plot for the same experiments; τ_c was calculated from the intensities of the aromatic resonances of Tyr-1, as described in the text

plateau at lipid concentrations at which τ for the human peptide is still changing. It should be noticed in Fig. 6B that τ_c for Tyr-1 is already larger for the camel than for the human analog even *in the absence of lipids* (ca. 2.4 vs 1.6 ns). This may suggest a more compact structure, or a closer involvement of the N-terminal side chain with the rest of the molecule in the camel analog.

Acknowledgements. This work was supported by the Progetto Finalizzato Chimica Fine e Secondaria of the Italian CNR (Sottoprogetto: Cc) and is part of a binational program (Italy/The Netherlands).

References

Bolognesi M, Gatti G, Menegatti E, Guarneri M, Marquart M, Papamokos E, Huber R (1982) Three dimensional structure of the complex between pancreatic secretory trypsin inhibitor (Kazal type) and trypsinogen at 1.8 Å resolution. Structure solution, crystallographic refinement and preliminary structural interpretation. J Mol Biol 162: 839–868

De Marco A, Wüthrich K (1976) Digital filtering with a sinusoidal window function: an alternative technique for resolution enhancement in FT NMR. J Magn Reson 24: 201–204

De Marco A, Menegatti E, Guarneri M (1979) ^{1}H nuclear-magnetic-resonance studies of the porcine pancreatic secretory trypsin inhibitor at 270 MHz. Eur J Biochem 102: 185–194

De Marco A, Menegatti E, Guarneri M (1982a) pH and temperature effects on the molecular conformation of the porcine pancreatic secretory trypsin inhibitor as detected by hydrogen-1 nuclear magnetic resonance. Biochemistry 21: 222–229

De Marco A, Menegatti E, Guarneri M (1982b) ^{1}H-NMR studies of the structure and stability of the bovine pancreatic secretory trypsin inhibitor. J Biol Chem 257: 8337–8342

Ferrige AG, Lindon JC (1978) Resolution enhancement in FT NMR through the use of a double exponential function. J Magn Reson 31: 337–340

Gibbons WA, Beyer CF, Dadok J, Sprecher RF, Wyssbrod HR (1975a) Studies of individual amino acid residues of the decapeptide tyrocidine A by proton double-resonance difference spectrocopy in the correlation mode. Biochemistry 14: 420–429

Gibbons WA, Crepaux D, Delayre J, Dunand JJ, Hajdukovic G, Wyssbrod HR (1975b) The study of peptides by INDOR, difference NMR and time-resolved double resonance techniques. In: Walter R, Meienhofer J (eds) Peptides: Chemistry, structure and biology. Ann Arbor Science Publishers, Ann Arbor, MI, pp 127–137

Hammonds RG Jr, Nicolas P, Li CH (1982) β-Endorphin: analgesic and receptor binding activity of non-mammalian homologs. Int J Pept Protein Res 19: 556–561

Hore PJ, Egmond MR, Edzes HT, Kaptein R (1982) Cross-relaxation effects in the photo-CIDNP spectra of amino acids and proteins. J Magn Reson 49: 122–150

Jarrel HC, Deslaurier R, McGregor WH, Smith JPC (1980) Interaction of opioid peptides with model membranes. A carbon-13 nuclear magnetic study of enkephalin binding to phosphatidylserine. Biochemistry 19: 385–390

Kaptein R (1982) Photo-CIDNP studies of proteins. In: Berliner LJ, Reuben J (eds) Biological magnetic resonance, Vol 4. Plenum Press, New York, p 145

Lanir A, Navon G (1971) Nuclear magnetic resonance studies of bovine carbonic anhydrase. Binding of sulfonamides to the zinc enzyme. Biochemistry 10: 1024–1032

Marion D, Wüthrich K (1983) Application of phase sensitive two-dimensional correlated spectroscopy (COSY) for measurements of ^{1}H-^{1}H spin-spin coupling constants in proteins. Biochem Biophys Res Commun 113: 967–974

Pachler KGR (1964) NMR study of some α-amino acids. II: Rotational isomerism. Spectrochim Acta 20: 581–587

Wagner G, De Marco A, Wüthrich K (1976) Dynamics of the aromatic amino acid residues in the globular conformation of the basic pancreatic trypsin inhibitor (BPTI). I: ^{1}H NMR studies. Biophys Struct Mech 2: 139–158

Zetta L, Kaptein R, Hore PJ (1982) A photo-CIDNP investigation of tyrosine mobility and exposure in human β-endorphin in the presence of phospholipid micelles. FEBS Lett 145: 277–280

Zetta L, Hore PJ, Kaptein R (1983) Investigation by photochemically induced dynamic nuclear polarization and nuclear Overhauser enhancement ^{1}H-NMR of the interaction between β-endorphin and phospholipid micelles. Eur J Biochem 134: 371–376