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Fluorescence Studies on the Conformation of Litorin in Solution and in the Presence of Model Membranes

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The conformation of the nonapeptide hormone litorin has been studied in buffer and in the presence of lipids, using static and dynamic fluorescence. The results obtained show that, in buffer, the hormone probably exists in a collection of flexible conformers, slowly interconverting between them. The marked changes observed in fluorescence spectra and lifetimes upon addition of dimyristoylphosphatidylserine vesicles clearly show that the peptide interacts with lipids assuming lipid specific conformations. Interestingly, no significative spectroscopic changes are produced by exposure to dimirystoylphosphatidylcholine vesicles both in the gel and liquid-christalline phases, suggesting a requirement for negatively charged lipids during the process of hormone-membrane interaction.

KEY WORDS: Litorin; bombesin-like peptides; fluorescence spectroscopy; membranes.

INTRODUCTION

The nonapeptide hormone litorin, which has the sequence pGlu–Gln–Trp–Ala–Val–Gly–His–Phe–Met–NH_2, belongs to the family of bombesin-like peptides (BLP) and shares with them a wide spectrum of biological activities [1]. Because such activities are similar but not identical, it has been suggested that BLP could interact with various receptor subtypes [2]. The knowledge of the structure of the hormones both in solution and in the presence of the lipidic environment in which the receptor is embedded is therefore of paramount importance for understanding the hormone–receptor interaction. Fluorescence spectroscopy should provide a useful tool for this purpose, as BLP contain a single tryptophan residue which can serve as an intrinsic probe for peptide conformation and dynamics [3]. In previous reports we have evaluated the structural characteristics of bombesin [4] and of gastrin releasing peptide (GRP) [5] using both static and time-resolved fluorescence. Some preliminary results on litorin are presented here to compare its conformational behavior with that of related peptides.

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MATERIALS AND METHODS

Crystalline litorin was purchased from Sigma (St. Louis, MO) and its purity was assayed by high-performance liquid chromatography. Dimyristoylphosphatidylserine (DMPS) and dimyristolphosphatidylcholine (DMPC) were obtained from Avanti Polar Lipids (Birmingham, AL) and used without further purification. All other chemicals were of analytical-grade purity.

Peptide concentration was determined spectrophotometrically using ϵ_{280} = 6000 L cm^{-1} mol^{-1}. DMPS and DMPC vesicles were prepared as described previously [5].

Absorption and time-averaged fluorescence measurements were performed using a Varian DMS 200 spectrophotometer and a SLM 8000 spectrophotofluorometer, respectively. For time-resolved fluorescence

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Table I. Fluorescence Parameters of 10^{-5} *M* Litorin in Buffer, pH 6, and in the Presence of DMPS Vesicles[a]

	τ_1 (ns) ±0.03	τ_2 (ns) ±0.03	τ_3 (ns) ±0.02	τ_4 (ns) ±0.01	α_1 ±0.01	α_2 ±0.01	α_3 ±0.01	α_4 ±0.01	SVR
Litorin	2.05	1.15	0.34	0.03	0.27	0.28	0.17	0.28	2.03
Litorin + DMPS	3.91	1.94	0.67	0.14	0.13	0.34	0.32	0.21	1.95

[a] τ_i, fluorescence lifetimes; α_i, normalized preexponential factors. Excitation wavelength, 295 nm; emission wavelength, 340 nm. SVR: serial variance ratio, equal to 2 for an ideal fit.

measurements, the excitation source was a Spectra Physics synchronously pumped, cavity-dumped dye laser system. The decay of the fluorescence intensity, $I(t)$, was analyzed in terms of a sum of exponentials according to the following relation:

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i}$$

where the preexponential terms, α_i, and the decay constants, τ_i, are the parameters of the decay function. The experimental curves were analyzed with a nonlinear reconvolution procedure described previously [6] and implemented to analyze multiple decay curves simultaneously [7]. This method was also used to resolve the decay-associated spectra (DAS). All measurements were carried out at 20±1°C.

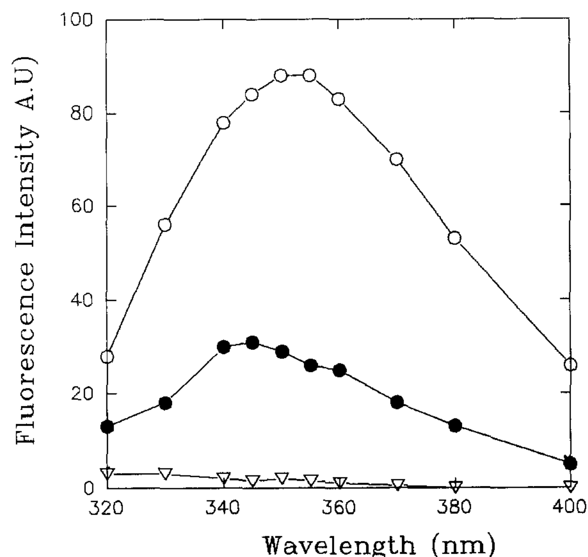

Fig. 1. Fluorescence DAS of 10^{-5} *M* litorin in 10 m*M* cacodilate, pH 6: (○) 2.05 ns, (●) 1.15 ns, and (▽) 0.34 ns. Excitation wavelength, 295 nm.

RESULTS

The fluorescence spectrum of litorin in aqueous solution has a maximum at 350 nm and this wavelength is not affected by pH in the range 3–9. The fluorescence quantum yield and the time-averaged fluorescence anisotropy are, respectively, 0.059±0.001 and 0.025±0.002 at pH 7 and do not change with the peptide concentration in the tested range (3×10^{-5}–10^{-6} *M*). However, at pH values higher than 7 and at concentrations near 5×10^{-5} *M* the peptide begins to aggregate.

The decay of the fluorescence intensity of litorin in buffer was measured at pH 6, where the peptide is supposed to be in the monomeric form, and the fluorescence parameters values are shown in Table I. The fluorescence decay is best described by a sum of four exponentials, where the addition of the shortest component provides a significant improvement in the statistical parameters with respect to a three-exponential fit.

The DAS for litorin at pH 6 are reported in Fig. 1. The spectrum associated with the longer lifetime shows a fluorescence maximum near 350 nm, typical of a fully exposed Trp residue, while the intermediate ones are slightly blue shifted; the contribution of the shortest lifetime is too low to be resolved.

The effect of DMPS on the fluorescence of litorin at pH 6 is depicted in Fig. 2. The addition of aliquots of a concentrated solution of the lipid to a litorin sample causes a progressive, saturable increase in the fluorescence intensity. The emission maximum wavelength and the time-averaged fluorescence anisotropy at lipid saturation are 336±1 nm and 0.15±0.01, respectively.

The apparent binding contrast, calculated from the wavelength shift, as suggested by Deber and Benham [8], is $K_a = 2.5\times10^4\ M^{-1}$.

The decay of the fluorescence intensity of litorin at pH 6 in the presence of a saturating amount of DMPS was measured and the parameters values are shown in Table I. All the lifetimes increase in parallel with the fluorescence intensities with respect to the values in buffer.

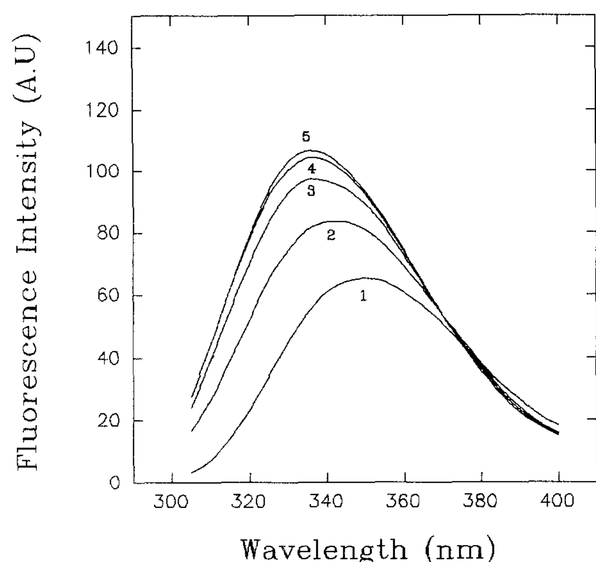

Fig. 2. Fluorescence titration of litorin with DMPS: (1) 10^{-5} *M* litorin in 10 m*M* cacodilate, pH 6; (2) DMPS-to-litorin ratio L/P = 8; (3) L/P = 19.2; (4) L/P = 38.4; (5) L/P = 54.5.

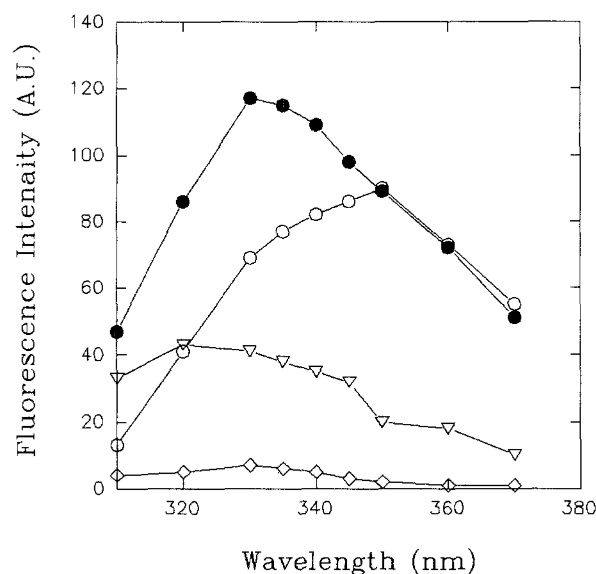

Fig. 3. Fluorescence DAS of 10^{-5} *M* litorin in 10 m*M* cacodilate, pH 6, in the presence of 7×10^{-4} *M* DMPS: (○) 3.91 ns, (●) 1.94 ns, (▽) 0.97 ns, and (◇) 0.14 ns. Excitation wavelength, 295 nm.

The DAS are depicted in Fig. 3 and show remarkable differences from the case of litorin alone. In fact, the contribution of the spectrum associated to the lifetime of 1.94 ns becomes predominant and the shortest-lifetime component gives an appreciable contribution to the total fluorescence.

Differently from DMPS, the addition of DMPC vesicles does not cause any detectable change in the hormone fluorescence. Furthermore, experiments carried out at 50°C, where both DMPS and DMPC are in the liquid crystalline phase, do not show substantial differences from those performed at 20°C.

DISCUSSION

The fluorescence maximum wavelength at 350 nm and the fluorescence anisotropy close to zero clearly indicate that the Trp residue is exposed to the solvent and experiences a high degree of mobility. The circular dichroism spectrum (not shown) under the same conditions is typical of a random coil conformation. The tendency to aggregate at a pH higher than 7 is probably due to an increase in hydrophobicity of the peptide as a consequence of the deprotonation of the His residue.

The fluorescence decay shows that at least four conformers, stable on the nanosecond time scale, are present at the same time in solution, slowly interconverting between them. Probably, the spectra associated to the shorter lifetimes correspond to less extended configurations where the Trp fluorescence can be easily quenched from other groups.

Fluorescence experiments are very informative in characterizing the interaction of the hormone with model membranes. The blue shift of the emission maximum and the increase in fluorescence intensity clearly indicate that the portion of the peptide containing the Trp residue has to be partially shielded from water. Moreover, the larger fluorescence anisotropy of litorin in DMPS, compared to litorin in buffer, suggests a partial immobilization of the indole ring in the lipid matrix.

Litorin binds with a high apparent binding constant to the negative charged lipids but does not interact with neutral vesicles either in gel or in the liquid crystalline phase. The results are similar to those found for the related peptides bombesin and GRP [5,9]. This behavior indicates that negative lipids play an important role in the BLP–receptor interaction by accumulating the peptide on the membrane surface via an electrostatic mechanism, as suggested by Schwyzer [10]. The DAS of litorin in the presence of DMPS show dramatic changes from those in water. This probably indicates that the lipids can stabilize particular conformations of the peptide sequence. This conclusion is confirmed from circular dichroism experiments (data not shown) which suggest that the peptide can assume, in contact with lip-

ids, a secondary structure similar to a β-sheet. At the contrary, an α-helix-like structure was induced in bombesin and GRP under the same conditions.

In conclusion, the fluorescence results strongly support the idea that litorin, and in general all the BLP family, interacts with a negatively charged environment on the membrane surface, assuming different conformations. One of these conformers could drive the hormone to interact correctly with the appropriate receptor subtype. Such a mechanism has been exhaustively demonstrated in the case of opioid hormones [10].

REFERENCES

1. V. Erspamer, and P. Melchiorri (1983) in E. E. Muller and R. M. McLeod (Eds.), *Neuroendocrine Perspectives, Vol. 2*; Elsevier, New York, pp. 37–106.
2. L. Negri, G. Improta, M. Broccardo, and P. Melchiorri (1988) in Y. Taché, P. Melchiorri, and L. Negri (Eds.), *Bombesin-like Peptides in Health and Disease*, Ann. NY. Acad. Sci., Vol. 547, pp. 415–428.
3. A. P. Demchenko (1992) in J. R. Lakowicz (Ed.), *Topics in Fluorescence Spectroscopy, Vol. 3, Biochemical Applications*, Plenum Press, New York, pp. 65–111.
4. P. Cavatorta, A. Spisni, A. G. Szabo, G. Farruggia, L. Franzoni, and L. Masotti (1989) *Biopolymers* **28,** 441–463.
5. P. Cavatorta, G. Sartor, P. Neyroz, G. Farruggia, L. Franzoni, A. G. Szabo, and A. Spisni (1991) *Biopolymers* **31,** 653–661.
6. M. Zuker, A. G. Szabo, L. Bramall, D. T. Krajcarski, and B. Selinger (1985) *Rev. Sci. Instrum.* **56,** 14–18.
7. J. R. Knutson, J. M. Beechem, and L. Brand (1985) *Chem. Phys. Lett.* **102,** 501–507.
8. C. M. Deber and B. A. Benham (1985) *Biopolymers* **24,** 105–116.
9. P. Cavatorta, G. Farruggia, L. Masotti, G. Sartor, and A. G. Szabo (1986) *Biochem. Biophys. Res. Comm.* **141,** 99–105.
10. R. Schwyzer (1986) *Biochemistry* **25,** 6335–6342.