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Role of Negative Charge on Acidic Lipids in the Interaction with Human Growth Hormone-Releasing Factor

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It was proved that a negative charge on acidic lipid was indispensable for the interaction between human growth hormone-releasing factor fragment (hGRF(1-29)NH_2) and lipid. The characteristic conformational changes of the peptide were observed by CD spectroscopy on addition of several acidic lipids, whereas such changes were not observed on addition of neutral lipids.

Human growth hormone-releasing factor (hGRF) consisting of 44 amino acids is one of hypothalamic hormones. It promotes secretion of growth hormone at pituitary glands. The primary structure-activity study has been investigated extensively,[1-6] but the real "active conformation" is still unknown. On the basis of two-stage capture model[7] or multiple sequential steps model,[8] several bioactive peptides in vivo reach cell membrane from plasma at first and interact with lipid with changing their conformation characteristic, before they reach the target receptor. Thus here, the interaction between hGRF(1-29)NH_2 and several lipids was investigated by analyzing the conformation of hGRF(1-29)NH_2 (Fig. 1), which possessed almost complete activity as compared with intact hGRF in vivo and in vitro,[1,2,5,6] using CD spectroscopy.

Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-NH_2

Fig. 1. Amino acid sequence of hGRF(1-29)NH_2.

hGRF(1-29)NH_2 was synthesized by the solid-phase method as reported previously.[1-4] Extractive or synthetic lipids were of commercial origin and were used without further purification. Size-ordered liposome was prepared by an Extruder, the diameter of which was found to be distributed sharply by a light scattering technique. CD spectra were recorded on a Jasco J-600 spectrometer and presented as mean residue molar ellipticity.

The addition of 1,2-dimyristoyl-sn-glycero-3-phosphorylglycerol (DMPG) liposome to hGRF(1-29)NH_2 in the aqueous solution (pH7.0) above the phase transition temperature of DMPG caused

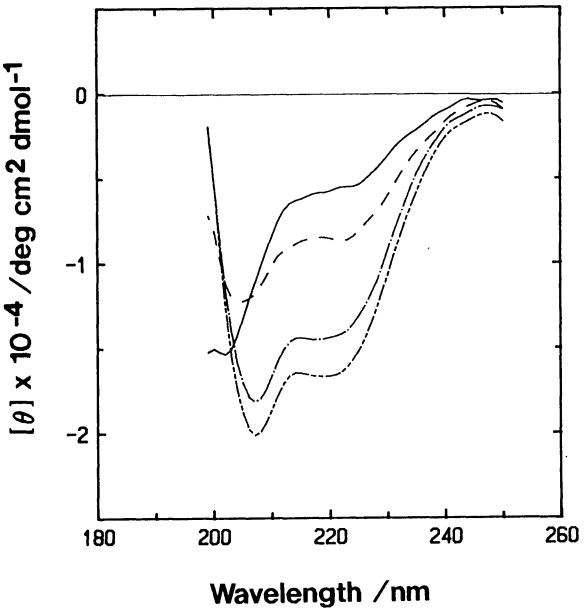

Fig. 2. CD spectra of 2.7 μM hGRF (1-29)NH_2 alone(——) or in the presence of 7.0 μM(– – –), 23 μM(—·—) and 115 μM(—···—) DMPG liposome.

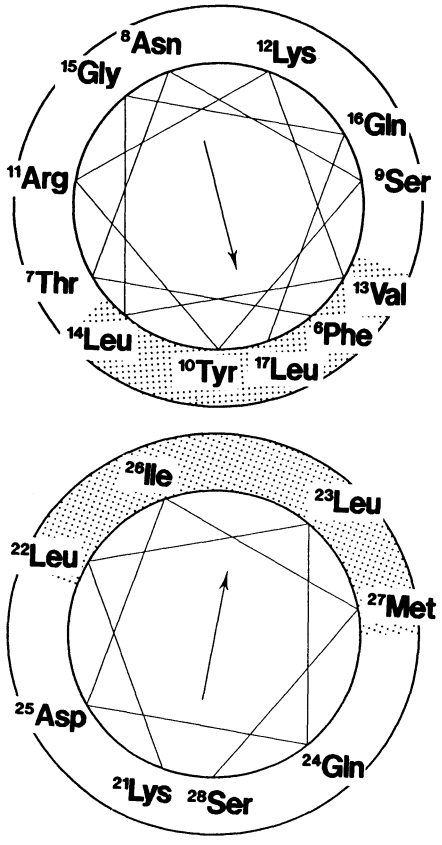

Fig. 3. Axial projections of hGRF (6-17) and (21-28); Screened areas and central arrows show hydrophobic surfaces and hydrophobic moments, respectively.

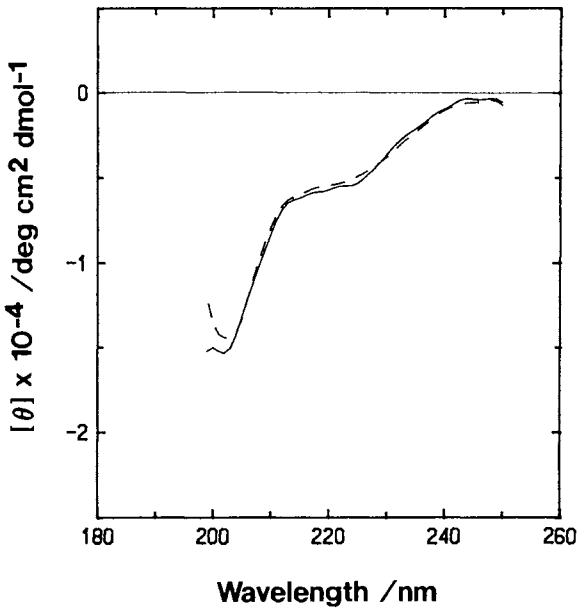

Fig. 4. CD spectra of 2.7 μM hGRF (1-29)NH_2 alone(——) or in the presence of 132 μM DMPC liposome(– – –).

remarkable conformational change, which is regarded as the increase of helix (Fig. 2). The helical content of the peptide in 115 μM (1 M = 1 mol dm^{-3}) DMPG calculated by CONTIN[9] is 66%. According to the axial projections of parts of hGRF(1-29)NH_2 shown in Fig. 3 indicates that this peptide would form amphiphilic helix. The hydrophobic moments[10] and the mean hydrophobicities[11] of hGRF(6-17) and (21-28) are 0.29, -0.23, 0.51, and -0.14, respectively. Therefore it is supposed that the hydrophobic surface of the peptide bounded the alkyl chains of DMPG by hydrophobic interaction. Similar conformational change was also observed by the addition of other acidic lipids such as phosphatidylserine or ganglioside.

On the other hand the additions of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) or egg yolk phosphatidylcholine caused no conformational change. The spectrum of hGRF(1-29)NH_2 in the presence of DMPC liposome, as shown in Fig. 4, was identical to that of the peptide alone for at least two days even after the treatment of annealing or sonication. Physical properties, such as cmc, phase transition temperature and phase morphology, of DMPG and DMPC are almost the same except charges on a head group. Thus, considering that hGRF(1-29)NH_2 is basic peptide, the results imply that a negative charge on acidic lipid is indispensable for the conformational change. In other words, the interaction between the peptide and lipids is constructed with not only a hydrophobic interaction but also an electrostatic interaction between positive charges on the peptide and negative charge on acidic lipid (Fig.

5).

Cell membrane of human pituitary glands includes acidic phospholipid as only 4% weight of total lipids.[12] However the results of this study suggests that minor acidic lipids play an important role in the interaction and transmission of hGRF in vivo.

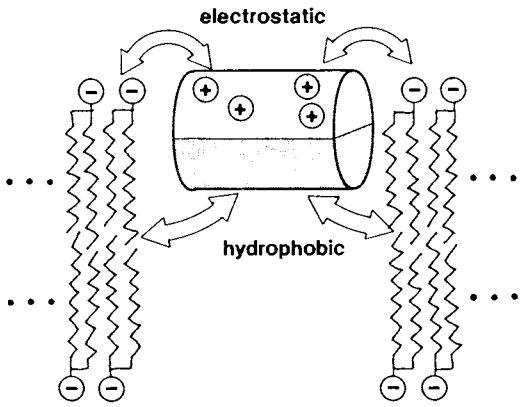

Fig. 5. Concept of the interaction between hGRF and acidic lipids.

References

1) S. Ohashi, M. Shiraki, S. Sawano, S. Ozaki, K. Akimoto, T. Takaoka, S. Hirose, and T. Kurihara, Pept. Chem. 1985, 23rd, 45 (1986).

2) S. Ohashi, M. Shiraki, S. Sawano, M. Seki, and S. Ozaki, Pept. Chem. 1987, 25th, 521 (1988).

3) S. Ohashi, T. Kokubu, S. Sawano, and H. Masuda, Pept. Chem. 1988, 26th, 79 (1989).

4) T. Kokubu, S. Ohashi, and S. Sawano, Pept. Chem. 1989, 27th, 103 (1990).

5) V. A. Lance, W. A. Murphy, J. Sueiras-Diaz, and D. H. Coy, Biochem. Biophys. Res. Commun., 119, 265 (1984).

6) N. Ling, A. Baird, W. B. Wehrenberg, N. Ueno, T. Munegumi, and P. Brazeau, Biochem. Biophys. Res. Commun., 123, 854 (1984).

7) T. Higashijima and T. Miyazawa, Tanpakushitsu Kakusan Koso, 29, 29 (1984).

8) D. F. Sargent and R. Schwyzer, Proc. Natl. Acad. Sci. U. S. A., 83, 5774 (1986).

9) S. W. Provencher and J. Glöckner, Biochemistry, 20, 33 (1981).

10) D. Eisenberg, R. M. Wiss, and T. C. Terwilliger, Nature, 299, 371 (1982).

11) J. Janin, Nature, 277, 491 (1979).

12) Singh and K. Carroll, Lipids, 5, 121 (1970).

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