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Synthesis, conformational and biological properties of lipophilic derivatives of gastrin and cholecystokinin peptides

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Abstract: The fully active [Nle^{15}]-little-gastrin-(2-17) and [Thr^{28},Nle^{31}]-CCK-(25-33) were lipo-derivatized by N-terminal incorporation of di-fattyacyl-glycerol moieties. Although the induced tight interdigitation of the lipo-tail with the lipid compartments of cell membrane bilayers prevent an escape of the lipophilic hormones into the extracellular water phase, they retain high receptor binding affinities. This confirms that a membrane-bound pathway for hormone receptor recognition is possible, even if it implies lateral penetration of the complex channel type suprastructure by the ligand at the lipid/water interface.

Introduction

Recognition of peptide hormones by membrane-bound receptors can occur either via collision of the hormone directly from the extracellular aqueous phase with the receptor's surface-exposed binding sites, or be mediated by the statistically more favored pre-adsorption on the target cell membrane. In this bimolecular interaction of the flexible peptide molecule with the membrane-bound receptor degrees of freedom of overall translation and rotation of the ligand are lost, and internal rotations about single bonds of the flexible peptide restricted, thus leading to an entropic penality which has to be compensated by enthalpy. It has therefore been proposed that the binding process occurs in successive steps by capture of the free ligand at the cell surface upon statistical collision, and by pre-orientation and pre-folding at the membrane bilayer followed by a membrane-bound two-dimensional migration to the receptor (1,2). Fast developments in molecular biology allowed recently for cloning and sequencing of a large number of membrane-bound receptors, the majority of which are composed of seven transmembrane segments connected at the bilayer surfaces by more or less extended loops forming relatively large boundary structures. Spatial models of these receptors (3,4) constructed in analogy to the known 3D-structure data of bacteriorhodopsin (5) and more properly of mammalian rhodopsin (6) are hardly compatible with lateral penetration of ligands as required for the hypothetical membrane-bound pathway of the hormone/receptor binding process shown in Fig. 1.

Using gastrin (HG) and cholecystokinin (CCK) as model peptide hormones, an answer to this open question was attempted by their lipophilic derivatization which should allow the membrane bilayer to sequester the ligands whithout possibility of escape into the water phase. Correspondingly, interaction of the hormone derivatives with the receptors should then occur exclusively at the water/lipid interface.

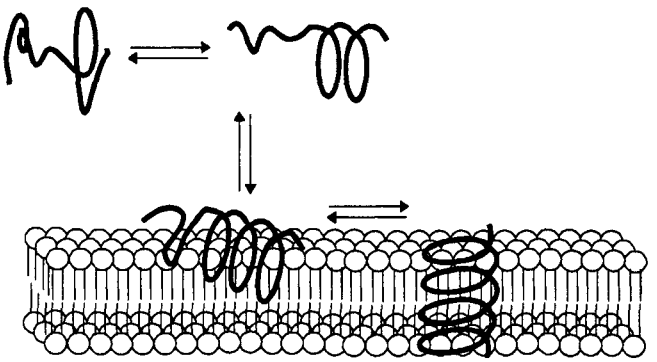
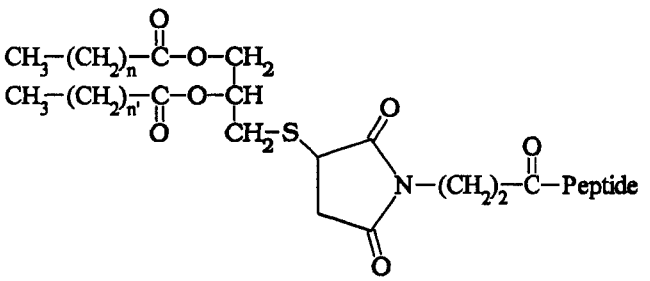


Figure 1. Partition equilibrium of peptide hormones at the cell membrane surface with concomitant pre-orientation and pre-folding prior to receptor recognition as proposed by Schwyzer (1,2).

Results

In order to assure tight interdigitation with membrane bilayers double-tailed lipid moieties were grafted to the peptide molecules in a fashion similar to that used by nature as membrane anchors for various types of proteins, e.g. glycosyl-phosphatidylinositol, or fatty acylation. Since N-terminal modifications of gastrin- and CCK-peptides are known to affect only marginally their bioactivity profiles, di-fattyacyl-mercaptoglycerol moieties (7) were linked covalently at these sequence positions via the thiol/maleimide reaction principle to produce the lipophilic adducts shown in Fig. 2 (8, 9).



Peptide: Arg-Asp-Tyr(SO_3)-Thr-Gly-Trp-Nle-Asp-Phe-NH_2 [Thr^{28},Nle^{31}]-CCK-(25-33)

Gly-Pro-Trp-Leu-$(Glu)_5$-Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH_2 [Nle^{15}]-gastrin-(2-17)

Figure 2. Chemical structure of DM-CCK and DM-gastrin (n=n'=12).

As expected from the chemical structure, the lipo-peptides aggregate spontaneously in aqueous solution into spherical vesicles upon extrusion or sonication in the case of DM-gastrin and by simple vortexing in the case of DM-CCK. Despite the sequence homology of the peptidic headgroups remarkably different vesicle systems are formed in terms of stability, but all were in the liquid state above 5°C (8, 9). Because of this relative fluidity of the vesicles the transfer of the lipo-peptides to phosphatidylcholine bilayers (DPPC and DMPC), as model membranes, occurs rapidly and quantitatively whereby DM-gastrin is inserted statistically into the lipid bilayer, whilst DM-CCK was found to form differently enriched DM-CCK domains (8, 9). As these CCK-enriched domains exhibit a higher affinity for Ca^{2+} ions than the rest

of the bilayer, a possible physiological relevance of this phenomenon was investigated by measuring Ca^{2+} influx into DMPC small unilamellar vesicles (SUV) as possibly induced by the lipo-peptides. Influx of Ca^{2+} could be observed as shown in Fig. 3, however, at rates which exclude a direct stimulation of Ca^{2+} release from reserves on cell membranes by the hormones, i.e. without implication of inositol(tris)phosphate as second messenger of the hormone receptor interaction. This was further confirmed by similar experiments performed on fibroblasts which are known not to contain gastrin or CCK receptors.

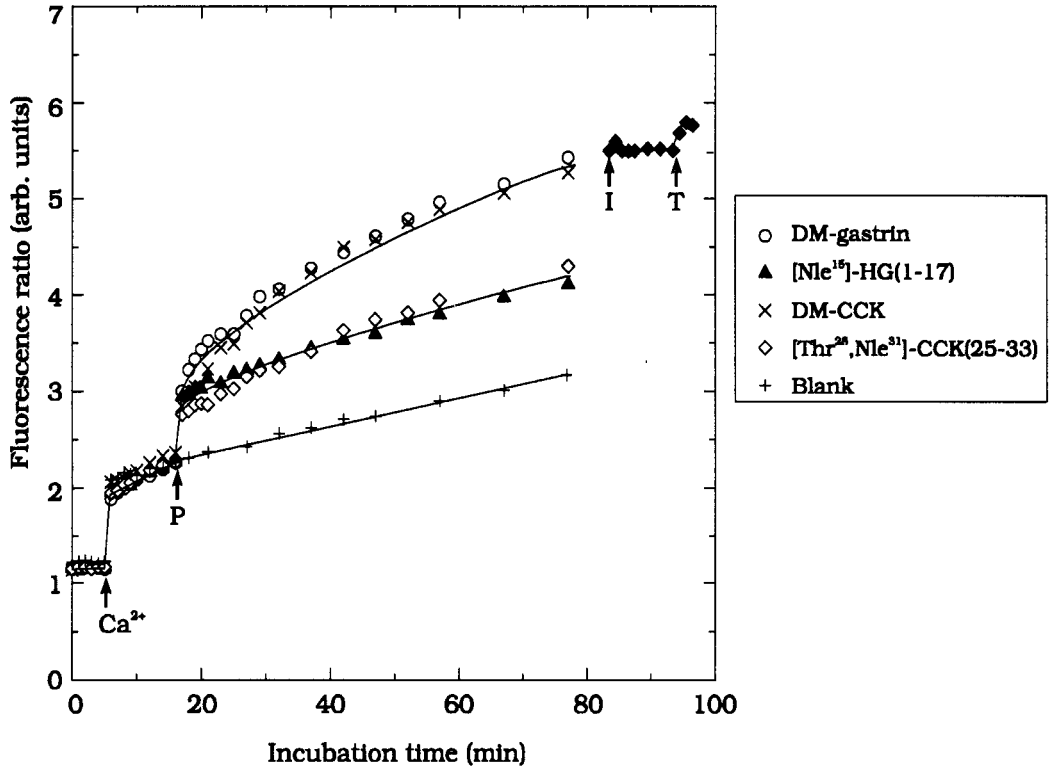


Figure 3. Dependence of the fluorescence ratio on incubation time of DMPC SUVs with entrapped fura-2 after the addition of [Thr^{28},Nle^{31}]-CCK-(25-33), [Nle^{15}]-gastrin-(1-17), DM-CCK and DM-gastrin. The buffer is used as blank.

Merging of DM-gastrin with lipid bilayers is accompanied by a conformational transition from a partly ordered to a predominantly unordered structure of the peptide moiety and to exposure of most of the gastrin to the bulk water (10). Conversely, the CCK-headgroup inserts its C-terminus into more hydrophobic compartments via an amphipatic helical array parallel to the interface with superimposed β-type hydrogen bonding pattern (11).

From the results of the model experiments an even faster net intervesicular transfer of the lipo-peptides to natural cell membranes was expected. In fact, the binding affinities of lipo-gastrin (8) and lipo-CCK (11) to receptors on AR4-2J and rat pancreatic acini, respectively, were only 7 and 5 to 6-fold lower than those of the parent hormones. These values compare well to those theoretically expected for the lower diffusion rate of a lipid monomer in lipid bilayers in respect to a molecule in water (11). It was further

confirmed by the strong effect of the fatty acid chain length (8) as well as by the observation that longer incubation periods leads to binding affinities almost identical to those of the parent hormone (11). Regarding the functional binding, lipo-gastrin was found to be less potent (5-fold) than the parent gastrin as determined in the aminopyrine uptake assay in isolated parietal cells (10), whereas lipo-CCK exhibited an about 100 times lower potency than the unmodified CCK-peptide in amylase release from pancreatic acini (11).

Conclusion

In view of these biological properties and of the fact that an escape of the double-tailed lipo-peptides into the extracellular water phase is energetically highly unfavored, a lateral penetration of the loop domains of the receptor by the peptide headgroup at the water/lipid interface has to take place since the spectroscopic measurements are locating in this section of model bilayers the lipo-peptides in a more (DM-CCK) or less (DM-gastrin) folded form. This would imply that a membrane-bound pathway in the hormone-receptor recognition process a priori is possible and that the loop domains exhibit sufficient flexibility and mobility to allow for lateral penetration of the peptide headgroups at the water/lipid interface to reach in a more or less efficient manner the binding cleft. As the lipid portion of the lipo-peptides remains associated to the bilayer compartment nearby the seven-helix boundle of the receptor, the different size of the spacer between the lipidic tail and the shortest fully active sequence of gastrin (12) and CCK (13) in lipo-gastrin and lipo-CCK, respectively, i.e. β-Ala-Gly-Pro-Trp-Leu-Glu vs. β-Ala Arg-Asp, could possibly account for the different degree of functional binding observed for the two peptides.

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