

MATERIALS AND METHODS FOR MAKING IMPROVED MICELLE COMPOSITIONS

This application is a continuation-in-part of International Application Number PCT/US98/14316, filed Jul. 9, 1998, which claims the priority benefit under 37 U.S.C. 119(e) of U.S. Provisional Application Ser. No. 60/052,078, filed Jul. 14, 1997.

BACKGROUND OF THE INVENTION

The present invention relates generally to biologically active compounds and more specifically to compounds, peptides, proteins, fragments, analogs, and modulators thereof which are amphipathic, i.e., have both hydrophilic and hydrophobic portions. Specifically, the invention relates to improved methods for the delivery and presentation of amphipathic peptides, proteins, fragments, analogs, and modulators thereof in association with micelles diagnostic, therapeutic, cosmetic and org, an, tissue and cell preservative uses. The invention also provides methods for the delivery of compounds that are insoluble in an aqueous solution. Specifically, the invention provide methods to produce sterically stabilized crystalline products comprised of a crystallized insoluble compound coated with a lipid surface.

Of particular interest to the present invention are the biologically active amphipathic peptides which are members of the family of peptide compounds including, but not limited to, vasoactive intestinal peptide (VIP), growth hormone releasing factor (GRF), peptide histidine isoleucine (PHI), peptide histidine methionine (PHM), pituitary adenylate cyclase activating peptide (PACAP), gastric inhibitory hormone (GIP), hemodermin, the growth hormone releasing hormone (GHRH), sauvagine and urotensin I, secretin, glucagon, galanin, endothelin, calcitonin, α_1 -proteinase inhibitor, angiotensin II, corticotropin releasing factor, antibacterial peptides and proteins in general, surfactant peptides and proteins, α -MSH, adrenomedullin, ANF, IGF-1, α_2 amylin, orphanin, and orexin. More specifically, the invention relates to improved therapeutic methods for delivering peptides in the VIP/GRF family of peptides, as well as other amphipathic peptides, to targeted tissues through use of improved micelle compositions comprising a member of the VIP/GRF family of peptides, amphipathic peptides in general, proteins, and biologically active analogues, fragments and modulators thereof.

VIP is a 28-amino acid neuropeptide which is known to display a broad profile of biological actions and to activate multiple signal transducing pathways. See, Said, *Peptides* 5 (Suppl. 1):149-150 (1984) and Paul and Ebadi, *Neurochem. Int.* 23:197-214 (1993). A Schiff-Edmundson projection of VIP as a π -helix reveals segregation of apolar and polar residues onto the opposite faces of the helix and that this amphipathic character is also evident when VIP is modeled as a distorted α -helix, which is reported in Musso, et al., *Biochemistry* 27:8147-8181 (1988). A correlation between the helix-forming tendency of VIP analogues and their biological activity is described in Bodanzky et al., *Bioorgan. Chem.* 3:133-140 (1974). In pure water, the spectral characteristics of VIP are consistent with those of a random coil. However, organic solvents and anionic lipids induce helical-information in the molecule. See, Robinson et al., *Biopolymers* 21:1217-1228 (1983); Hamed, et al., *Biopolymers* 22:1003-1021 (1983); and Bodanzky, et al., *Bioorganic Chem.* 3:133-140 (1974).

Short peptides capable of forming amphipathic helices are known to bind and penetrate lipid bilayers. See, Kaiser and

Kezdy, *Ann. Rev. Biophys. Biophysical Chem.* 15:561-581 (1987) and Sansom, *Prog. Biophys. Molec. Biol.* 55:139-235 (1991). Examples include model peptides like (LKKLLKL-), which are disclosed in DeGrado and Lear, *J. Am. Chem. Soc.* 107:7684-7689 (1985), and the 26-residue bee venom peptide, melittin, disclosed in Watata and Gwozdinski, *Chem-Biol. Interactions* 82:135-149 (1992). Possible mechanisms for the binding include alignment of peptide monomers parallel to the surface of the bilayer mediated by electrostatic interactions between polar amino acids and phospholipid head groups, and insertion of peptide aggregates into the apolar bilayer core, stabilized in part, by the hydrophobic effect. See, Sansom, *Prog. Biophys. Molec. Biol.* 55:139-235 (1991).

VIP belongs to a family of homologous peptides, other members of which include peptide histidine isoleucine (PHI), peptide histidine methionine (PBM), growth hormone releasing factor (GRF), pituitary (GIP), hemodermin, the growth hormone releasing hormone (GHRH), sauvagine and utotensin I, secretin and glucagon. Like VIP, the other members of the VIP/GRF family of peptides, and biologically active analogues thereof, can form amphipathic helices capable of binding lipid bilayers. The biological action of members of the VIP/GRF family of peptides are believed to be mediated by protein receptors expressed on the cell surface and intracellular receptors and it has recently been demonstrated that calmodulin is likely to be the intracellular receptor for VIP [Stallwood, et al., *J. Bio. Chem.* 267:19617-19621 (1992); and Stallwood, et al., *FASEB J.* 7:1054 (1993)].

Bodanzky et al., *Bioorgan. Chem.* 3:133-140 (1974) were the first to study the conformation of VIP through optical rotary dispersion and circular dichroism spectrum. They showed structural differences in VIP, depending on the hydrophobicity of the solvent in which VIP was dissolved. The VIP-in-water spectrum revealed a mostly random coil structure (about 80%). However, addition of organic solvents, such as trifluoroethanol (TFE) or methanol, even at low concentration induced a pronounced shift to a helical structure. The authors suggested that these effects of the organic solvents on the structure of the peptide would coincide with receptor conditions, and therefore, the helical conformation of VIP would correspond to an "active architecture" required for its biological activity. These early studies were in agreement with the findings of Robinson et al., *Biopolymers* 21:1217-1228 (1982), who analyzed the conformation of VIP, and two of its family members, secretin and glucagon, in water, anionic detergents, and anionic lipids (PA and phosphatidylglycerol (PG)). They showed an increase in the helix formation probability by arginyl, histidyl, and lysil residues, corresponding in all three peptides to their 13-20 amino acid region. A predominantly disordered structure was again observed for VIP in aqueous solvents, and zwitterionic lipids, suggesting that the charge of the polar head group plays an important role in helix formation. Using circular dichroism (CD) spectra studies with 40% HFIP/H₂O mixture and ¹H-NMR studies Fournier et al., *Peptides* 5:160-177 (1984), showed that the 15-28 portion of the VIP segment forms an α -helix in the presence of organic solvent. A complete structural study of the native VIP in 40% TFE was performed by Theriault et al., *Biopolymers* 31:459-464 (1991) using two-dimensional ¹H-NMR spectroscopy. Their results were similar to the ones obtained by Fry et al., *Biochemistry* 28:2399-2409 (1989) who investigated VIP in 25% methanol/water. They described two helical segments between the amino acids 7-15 and 19-27 linked by a random coil peptide chain portion that granted mobility to the molecule.