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CD and ^{31}P NMR studies of tachykinin and MSH neuropeptides in SDS and DPC micelles

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ABSTRACT

Secondary structural characteristics of substance P (SP), neurokinin A (NKA), neurokinin B (NKB), α-melanocyte stimulating hormone peptide (α-MSH), γ_1-MSH, γ_2-MSH, and melittin were evaluated with circular dichroism in phosphite buffer, DPC micelles, and SDS micelles. CD spectral properties of γ_1-MSH and γ_2-MSH as well as ^{31}P NMR of DPC micelles with all the peptides are reported for the first time. Although, a trend in the neuropeptide/micelle CD data appears to show increased α-helix content for the tachykinin peptides (SP, NKA, NKB) and increased β-sheet content for the MSH peptides (α-MSH, γ_1-MSH, γ_2-MSH) with increasing peptide charge, the lack of perturbed ^{31}P NMR signals for all neuropeptides could suggest that the reported antimicrobial activity of SP and α-MSH might not be related to a membrane disruption mode of action.

1. Introduction

Neuropeptides and antimicrobial peptides (AMPs) are often similar in size and charge [1–8]; since a few neuropeptides have reported antimicrobial activity, some authors suggest that these neuropeptides might be utilized in the body for both signaling and antimicrobial activities [9–17]. The central nervous system might be employing neuropeptides to perform antimicrobial activities to help the host protect itself against infection from various microbial organisms [9–16]. Substance P of the tachykinin family as well as α-melanocyte stimulating hormone peptide (α-MSH) of the MSH peptide family are examples of neuropeptides with reported antimicrobial activity [8,9,15,18–24]. Since many antimicrobial peptides and neuropeptides act at the cell surface [1,4,8,9,25–28], we were interested in biophysically characterizing the interaction of tachykinins and MSH peptides with model membranes. We investigated the interactions of selected tachykinin and MSH peptides with micelle model membranes via circular dichroism (CD) and ^{31}P nuclear magnetic resonance (NMR) spectroscopies. Circular dichroism was used to evaluate secondary structural characteristics of the tachykinin and MSH peptides in mimicked cerebrospinal fluid and membrane environments, while solution-state ^{31}P NMR was used to probe effects of peptides on the phospho head-groups of a micelle model-membrane. Since some antimicrobial peptides exhibit α-helix secondary structure, model-membrane disruption, and antimicrobial activity [25], we set out to assess if there is any correlation between neuropeptide secondary structure (CD data) and perturbations of micelle phospho head-groups (^{31}P NMR data).

Neurokinin A (NKA), neurokinin B (NKB), and substance P (SP) are tachykinin neuropeptides, which are found in the central and peripheral nervous systems, control contractions of vessels in

Abbreviations: (AMP), antimicrobial peptide; (CD), circular dichroism; (SDS), sodium dodecyl sulfate; (DPC), dodecylphosphocholine; (NMR), nuclear magnetic resonance; (MSH), melanocyte stimulating hormone.

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smooth muscles, and induce placental vasodilation, as well as share four conserved residues at the C-terminus (Phe-X-Gly-Leu-Met-NH_2, Table 1) [1,24,29–36]. The MSH peptides (α-MSH, γ_1-MSH, and γ_2-MSH) have regulatory activities related to cardiovascular regulation, the sympathetic nervous system, the inflammation response, and neuromuscular regeneration; they also share six conserved residues near the N-terminus (Tyr-X-Met-X-His-Phe-Arg-Trp, Table 1) [1,37–42]. Since the tachykinin and MSH peptides are neuropeptides, we chose conditions that mimicked the cerebrospinal fluid using a phosphite buffer at p*H* 7.3 with physiological sodium, chloride, potassium, and calcium ion concentrations [43–45]. We employed commonly used micelles as membrane mimics, sodium dodecyl sulfate (SDS, negatively charged) and dodecylphosphocholine (DPC, zwitterionic), so that our results can be readily compared to other micelle model-membrane studies. Individual CD studies are reported for SP, NKA, NKB, and α-MSH under varying conditions, but not for γ_1-MSH and γ_2-MSH, while no reports that directly compared peptides within a family were found. Given the reported antimicrobial activity of SP and α-MSH, we included a canonical antimicrobial peptide, melittin (Mel), in our CD and ^{31}P NMR studies for reference [46–48]. SP, NKA, NKB, α-MSH, γ_1-MSH, γ_2-MSH, and Mel were evaluated with circular dichroism in a phosphite buffer that mimicked cerebrospinal fluid and micelles that mimicked a membrane environment (DPC and SDS), while ^{31}P NMR was used to gather information about the phospho head-groups of DPC micelles in the presence of these peptides. Herein, we report and discuss our results for assessing a potential correlation between neuropeptide secondary structure (CD data), perturbations of micelle phospho head-groups (^{31}P NMR data), and reported antimicrobial activity.

2. Materials and methods

2.1. Materials

Dodecylphosphocholine (DPC) was obtained from Avanti Polar Lipids (Birmingham, AL). Sodium dodecyl sulfate (SDS) was obtained from Hoefer Incorporated (San Francisco, CA). Sodium hydrogenphosphite (Na_2HPO_3) was obtained from MP Biomedicals (Solon, OH). SP, NKA, NKB, α-MSH, and γ_2-MSH were obtained from Bachem America Incorporated (Torrance, CA), while melittin and γ_1-MSH were obtained from Polypeptide (France).

2.2. Buffer and Micelle Preparation

Mimicked cerebrospinal fluid was composed of a 25 mM phosphite buffer at p*H* 7.3 with approximate physiological sodium, chloride, potassium, and calcium ion concentrations (140 mM Na^+, 100 mM Cl^-, 5 mM K^+, 2 mM Ca^{2+}) [43–45]. This phosphite buffer was made by combining Na_2HPO_3 (157.4 mg), $CaCl_2$ (11.1 mg), KCl (18.65 mg), deionized water, adjusting to a p*H* of 7.3 ± 0.1 with HCl (~10 μL of 1 M HCl), and then adjusting the Cl^- and Na^+ concentrations with NaCl (~262.5 mg) to yield a final concentration of 100 mM Cl^-, 140 mM Na^+, with an approximate total volume of 50 mL. An Accumet Basic AB15 p*H* meter was used. This phosphite buffer was filtered (0.22 μm filter) and then stored at −20 °C. Sodium dodecyl sulfate (SDS) micelles were prepared by adding SDS (144.2 mg) to deionized water (50 mL) yielding 10 mM SDS (p*H* 7.0 ± 0.1), which is above the critical micelle concentration in water (8.2 mM) [51,52]. Dodecylphosphocholine (DPC) micelles were prepared by adding DPC (35.1 mg) to deionized water (50 mL) yielding 2 mM DPC (p*H* 6.2 ± 0.1), which is above the critical micelle concentration in water (1 mM) [51,53].

2.3. Circular dichroism

CD spectra (195–250 nm) were recorded using a JASCO J-815 circular dichroism spectropolarimeter. All spectra were recorded at room temperature (21 ± 1 °C) with quartz cuvettes of 1 mm path length. Spectra were obtained after an averaging of at least 50 scans. Background scans were taken with corresponding buffer or micelle preparation and subtracted from the peptide scans. Spectra were acquired with peptide concentrations between 75 and 100 μM in either the corresponding buffer or micelle solution. The instrument parameters were 0.1 nm data pitch, 1 s response time, 1.00 nm bandwidth, and 100 nm/min scanning speed. Due to high chloride concentration in the extracellular phosphite buffer, we were not able to scan below 195 nm.

2.4. ^{31}P NMR

Peptide-phospholipid interactions have been characterized with ^{31}P NMR; the ^{31}P signal broadens and/or shifts [54–57]. Peptides were in a lipid to peptide ratio of approximately 20:1 (2 mM DPC: 0.1 mM peptide) for all micelle samples. NMR measurements were taken at 121.9 MHz with a Bruker Avance 300 using a proton-decoupling pulse program. The 90° pulse time was 25 μs with a delay time of 5 s. D_2O (10%) was used as a solvent lock. All spectra were submitted to 5 MHz line broadening and referenced to 85% phosphoric acid in H_2O (0 ppm). Minor drift in the baseline of the spectra was corrected within the MATLAB program by fitting the sum of a polynomial and a Lorentzian function to the data; the Lorentzian function approximated the absorption and the polynomial approximated the baseline. The polynomial was subtracted from the data to yield a corrected spectrum.

3. Results and discussion

Tachykinin (SP, NKA, NKB) and MSH (α-MSH, γ_1-MSH, γ_2-MSH) neuropeptides were evaluated with circular dichroism in mimicked

Table 1
Sequences for neurokinin A (NKA), neurokinin B (NKB), substance P (SP), melanocyte stimulating hormone peptides (α-MSH, γ_1-MSH, γ_2-MSH), and melittin (Mel).

Peptide	Sequence	Length	Basic − acidic sites	Net charge at p*H* 7
tachykinin peptides				
NKA	HKTDSFVGLM-NH_2	10	+2	+1.1
NKB	DMHDFFVGLM-NH_2	10	0	−0.9
SP	RPKPQQFFGLM-NH_2	11	+3	+3.0
MSH peptides				
α-MSH	Ac-SYSMEHFRWGKPV-NH_2	13	+2	+1.1
γ_1-MSH	YVMGHFRWDRF-NH_2	11	+3	+2.1
γ_2-MSH	YVMGHFRWDRFG-OH	12	+2	+1.1
canonical AMP				
Mel	GIGAVLKVLTTGLPALISWIKRKRQQ-NH_2	26	+6	+6.0

Note: The net charge at p*H* 7 was estimated using side-chain pK_as [49,50].