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Using infrared spectroscopy of a nitrile labeled phenylalanine and tryptophan fluorescence to probe the α-MSH peptide's side-chain interactions with a micelle model membrane

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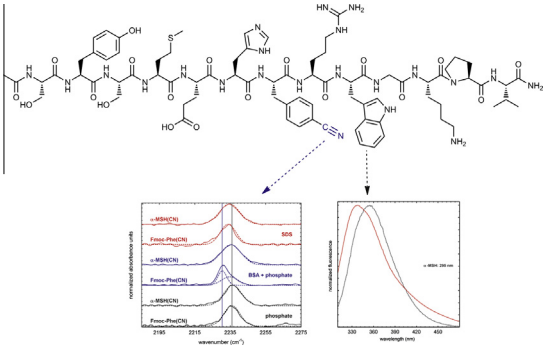
HIGHLIGHTS

- Trp fluorescence suggests that the Trp9 side-chain is buried in an SDS micelle.
- IR spectroscopy of Phe(CN)7 suggests that the Phe(CN)7 side-chain is not buried in SDS.
- Phe(CN)7 and Trp9 side-chains are not similarly interacting with an SDS micelle.
- IR data of a nitrile label complemented Trp fluorescence data quite well.

GRAPHICAL ABSTRACT



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ABSTRACT

The interactions of α-MSH (Ac-SYSMEHFRWGKPV-NH_2) side-chains were biophysically characterized with a micelle model membrane and in model intracellular bacterial conditions using infrared (IR) spectroscopy of a nitrile labeled α-MSH analogue, circular dichroism (CD), and tryptophan fluorescence. Local changes detected by the tryptophan and a nitrile-labeled phenylalanine using fluorescence and infrared spectroscopies, respectively, suggest that the Trp9 side-chain in the conserved core (HisPheArgTrp) of α-MSH is buried in an SDS micellar environment, while Phe(CN)7 does not appear to be buried.

1. Introduction

α-Melanocyte stimulating hormone peptide (α-MSH, Ac-SYSMEHFRWGKPV-NH_2, Fig. 1) is a neuropeptide found in humans with functions related to cardiovascular regulation, inflammation response, neuromuscular regeneration, as well as antimicrobial activity against *Staphylococcus aureus* [1–4]. Multiple authors suggest that some neuropeptides, possibly α-MSH, might also be utilized by the body for antimicrobial purposes [5–7]. Since neuropeptides and some antimicrobial peptides act at the cell surface, we biophysically characterized the interaction of α-MSH side-chains with a micelle model membrane. Given the unknown mode of antimicrobial action for α-MSH and that some antimicrobial peptides act within the cell [8,9], we also biophysically characterized the interaction of α-MSH side-chains in model intracellular bacterial conditions. Biophysical characterization of local or side-

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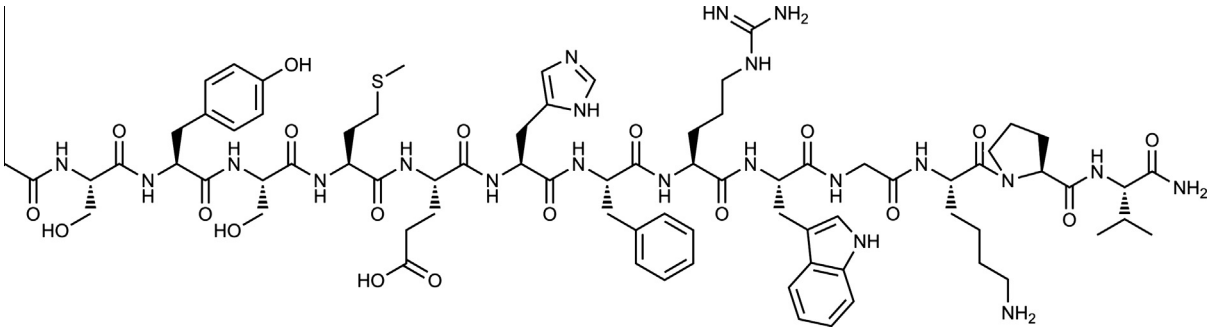

Fig. 1. Line drawing of α-MSH, Ac-SYSMEHFRWGKPV-NH_2.

chain interactions of α-MSH was accomplished with infrared (IR) spectroscopy of a nitrile labeled phenylalanine and tryptophan fluorescence, while global or secondary structure effects were characterized with circular dichroism (CD).

Several NMR, IR, and CD studies have explored the global conformation of α-MSH in aqueous and micelle/vesicle conditions [10–12]. These α-MSH studies offered varying conclusions. An NMR study explored the peptide in phosphate buffer (50 mM, p*H* 7.0) and concluded that it forms a hairpin loop in solution [10]. An IR study of the amide *I′* band, which explored α-MSH exposed to 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (10 mM, p*H* 7.4) and 1,2-dimyristoyl-glycero-3-phosphocholine/1,2-dimyristoyl-glycero-3-phosphoglycerol (DMPC/DMPG) vesicles, concluded that the peptide was largely random coil and β-sheet in buffer with slight alterations in negatively charged DMPG vesicles [11]. A CD study of α-MSH in phosphate buffer (40 mM, p*H* 7.4) and micelles/vesicles (SDS and DMPG) showed a slight conformational change in SDS/DMPG, which was attributed to α-MSH adopting a β-sheet like conformation [12]. Given that these studies employed slightly different conditions and techniques, slight variations in their specific conclusions about the global or secondary structural characteristics (hairpin loop, random coil/β-sheet, and β-sheet like) were not surprising. Of particular interest were Trp fluorescence studies of α-MSH with various vesicles (*e.g.* DMPG), which suggested that the Trp9 side-chain interacts with the lipids [13,14]. Consequently, we postulated that the nearby Phe7 side-chain also interacts with lipids, which was supported by previous modeling studies [15], and tested this hypothesis using a nitrile labeled Phe7 of α-MSH and IR spectroscopy. The use of the nitrile probe allows us to not only address this question, but also provides the opportunity to explore any potential phenomena or dynamics within a model of intracellular bacterial conditions (see Fig. 2).

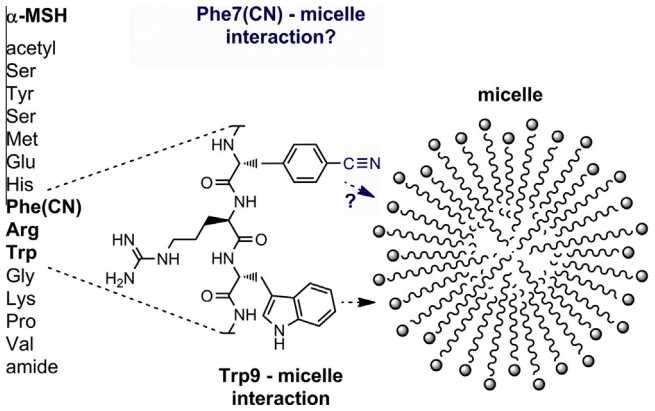

Fig. 2. Cartoon of the α-MSH Trp9 side-chain and potentially the Phe(CN)7 side-chain interacting with a micelle.

In this study, we employed a commonly used micellar membrane mimic, SDS (negatively charged), as well as buffering conditions that mimic the inside of an *Escherichia coli* (*E. coli*) cell [16–21]. Toward the latter aim, we also attempted to mimic any intracellular crowding effects by using a high concentration of bovine serum albumin (BSA) [22]. Since BSA chromophores would obscure the α-MSH chromophores in the CD or Trp fluorescence measurements, we used a nitrile labeled α-MSH and IR spectroscopy to probe any potential crowding effects. The utility of nitrile-labeled amino acids and IR to monitor local environmental changes of peptides has been successfully demonstrated by multiple groups [23–48]; additionally, others have used a similar label (thiocyanate, R—S—C≡N) in peptides [49–54]. Furthermore, tryptophan fluorescence readily provides environmentally dependent side-chain information of the indole ring [55,56]. Consequently, we used Trp fluorescence and IR spectroscopy to obtain side-chain specific (or local) information, while CD data was used to probe the potentially perturbative effect of nitrile labeling on α-MSH's global structure. With these techniques, Trp and Phe side-chains of the conserved HisPheArgTrp core in α-MSH were investigated.

2. Materials and methods

2.1. Materials

Except tyrosine, tryptophan, serine, arginine, and valine, Fmoc-amino acids and a ninhydrin test kit were obtained from AnaSpec Incorporated (Fremont, CA). Fmoc-Tyr(tBu)-OH was obtained from NovaBiochem (Hohenbrunn, Germany). Fmoc-Trp(Boc)-OH and Phe(4-CN)-OH were obtained from Bachem Bioscience Inc. (Torrance, CA). Fmoc-Ser(tBu)-OH and 2-(6-chloro-1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HCTU) were obtained from Peptides International (Louisville, KY). Fmoc-Arg(Pbf)-OH was obtained from CreoSalus (Louisville, KY). Fmoc-Val-OH was obtained from Advanced ChemTech (Louisville, KY). Rink amide AM resin, 4-methylpiperidine, triisopropylsilane ($(iPr)_3SiH$), sodium chloride and tryptophan were purchased from Acros Organics (Morris Plains, NJ). Trifluoroacetic acid (TFA) was purchased from Chem-Impex International (Wood Dale, IL). Diisopropylethylamine (DIEA), hydrochloric acid, calcium chloride, potassium chloride, *N,N*-dimethylformamide (DMF), dichloromethane (DCM), acetonitrile, and acetonitrile with 0.035% formic acid were obtained from Fisher Scientific (Fair Lawn, NJ). Sodium dodecyl sulfate (SDS) was obtained from Hoefer Incorporated (San Francisco, CA). α-MSH was obtained from Bachem (Torrance, CA). Fmoc-Phe(4-CN)-OH was obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and is abbreviated as Fmoc-Phe(CN) .