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Structural characterization of AS1–membrane interactions from a subset of HAMP domains

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ABSTRACT

HAMP domains convert an extracellular sensory input into an intracellular signaling response in a wide variety of membrane-embedded bacterial proteins. These domains are almost invariably found adjacent to the inner leaflet of the cell membrane. We therefore examined the interaction of peptides corresponding to either AS1 or AS2 of four different, well-characterized HAMP domains with several membrane model systems. The proteins included an *Archaeoglobus fulgidus* protein (Af1503), the *Escherichia coli* osmosensor $EnvZ_{Ec}$, the *E. coli* nitrate/nitrite sensor $NarX_{Ec}$, and the aspartate chemoreceptor of *E. coli* (Tar_{Ec}). Far-UV CD and NMR spectroscopy were used to monitor the induction of secondary structure upon association with neutral or acidic large unilamellar vesicles (LUVs) and bicelles. We observed significant increases in α-helicity within AS1 from $NarX_{Ec}$ and Tar_{Ec} but not in AS1 from the other proteins. To characterize these interactions further, we determined the solution structure of AS1 from Tar_{Ec} associated with acidic bicelles. The bulk of AS1 formed an amphipathic α-helix, whereas the N-terminal control cable, the region between TM2 and AS1, remained unstructured. We observed that the conserved prolyl residue found in AS1 of many membrane-adjacent HAMP domains defined the boundary between the unstructured and helical regions. In addition, two positively charged residues that flank the hydrophobic surface of AS1 are thought to facilitate electrostatic interactions with the membrane. We interpret these results within the context of the helix-interaction model for HAMP signaling and propose roles for AS1–membrane interactions during the membrane assembly and transmembrane communication of HAMP-containing receptors.

1. Introduction

HAMP domains (recently reviewed in [1]) are found adjacent to the inner leaflet of the cytoplasmic membrane within many prokaryotic membrane-spanning receptors [2], including well-characterized sensor histidine kinases (SHKs) and methyl-accepting chemotaxis proteins (MCPs) that serve as chemoreceptors. These domains have been identified within more than 5500 different proteins [2]. The first HAMP domain was identified by genetic analysis of the region joining the second transmembrane helix (TM2) of the serine chemoreceptor of *Escherichia coli* (Tsr_{Ec}) and the cytoplasmic domain responsible for signal propagation [3]. A subsequent bioinformatic analysis demonstrated that similar linker regions exist within four protein families: histidine kinases, adenlyate cyclases, methyl-accepting chemotaxis proteins and phosphatases [4]. Although HAMP domains are poorly conserved at the sequence level, they remain highly conserved at the level of secondary structure, consisting of two amphipathic sequences (AS1 and AS2) that form α-helices joined by a non-helical connector (CTR) [3,5,6].

Two models have been proposed for how HAMP domains receive sensory input from periplasmic or membrane-embedded stimulus-sensing domains. The crankshaft and gearbox model is based on the

Abbreviations: HAMP domain, domain present in histidine kinases, adenylyl cyclases, methyl-accepting proteins and phosphatases; SHK, sensor histidine kinase; MCP, methyl-accepting chemotaxis protein; TM1, first transmembrane helix; TM2, second transmembrane helix; AS1, first amphipathic sequence; AS2, second amphipathic sequence; AS1p, AS1-containing peptide; AS2p, AS2-containing peptide; CTR, connector domain; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol); LUV, large unilamellar vesicle; DHPC, 1,2-dihexanoyl-d_{22}-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-d_{54}-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-d_{54}-*sn*-glycero-3-phospho-(1′-*rac*-glycerol); Far-UV CD, Far-ultraviolet circular dichroism; PFG-NMR, Pulsed field gradient-nuclear magnetic resonance; TOCSY, Total correlation spectroscopy; NOESY, Nuclear Overhauser effect spectroscopy; PD1, periplasmic domain helix 1; PD4, periplasmic domain helix 4

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high-resolution structures of the HAMP domain of the *Archaeoglobus fulgidus* protein (Af1503) [7,8], which reveal that AS1 and AS2 of each subunit of a homodimeric protein form a parallel four-helix bundle. This model proposes that the C-terminal transmembrane helix (TM2) undergoes an axial rotation that causes a concerted rotation of AS1. The second model suggests that a small (~1–3 Å) piston-type displacement of TM2 perpendicular to the plane of the membrane occurs upon binding of a ligand to the periplasmic domain of various SHKs and chemoreceptors [9–17].

The second model has led to three different explanations for how these piston-type displacements are coupled to alterations in HAMP domain configuration. The first proposes that a piston-type displacement of a relatively rigid connection between TM2 and AS1 facilitates a scissor or pivoting-type motion within the HAMP domain [18]. The second, supported by extensive mutational evidence within an intact chemoreceptor (Tsr_{Ec}) [19–21], proposes that displacements of TM2 modulate the overall structural stability of the HAMP domain bundle via changing tension of a sequence connecting TM2 to AS1, referred to as the control cable [1,19,22]. The third explanation proposes that two HAMP conformations are in equilibrium: one in which the hydrophobic face of AS1 interacts with the inner leaflet of the membrane, and another in which AS1 interacts with AS2 [6]. However, none of these previous studies directly assessed the influence of the membrane on transmembrane communication by SHKs and chemoreceptors.

The work presented here was undertaken to determine whether AS1 is capable of association with a phospholipid bilayer. We chose four well-characterized HAMP domains and used circular dicroism (CD) and solution NMR to monitor the ability of their AS1 or AS2 peptides to interact with various model membrane systems. The *A. fulgidus* Af1503 protein was chosen because it was used for the high-resolution structures [7,8]. The *E. coli* EnvZ osmosensor ($EnvZ_{Ec}$) and the nitrate/nitrite-sensing histidine kinase ($NarX_{Ec}$) were selected because their HAMP domains have been extensively characterized [23–26]. Finally, the *E. coli* aspartate chemoreceptor (Tar_{Ec}) was analyzed because it is a well-characterized chemoreceptor [9–13,18] and extensive genetic studies have been performed with the closely related serine chemoreceptor Tsr_{Ec}, which serve as the basis for the dynamic bundle model of HAMP signaling [1,19–21].

2. Materials and methods

2.1. Materials

Synthetic peptides corresponding to either AS1 or AS2 (denoted AS1p and AS2p, respectively) were analyzed in the presence of different membrane model systems. The initial high-resolution three-dimensional structure of Af1503 HAMP (S278–V328) [7] served as the template for peptide design. Residues that form the flexible connector (G297–A309) were excluded from AS1p–Af1503 and AS2p–Af1503. For $EnvZ_{Ec}$, $NarX_{Ec}$ and Tar_{Ec}, similar peptides were constructed based on previously published [7,23,27] residue alignments. The resulting eight peptides are shown in Table 1. They were obtained from PolyPeptide Group (Strasbourg, France) and used without further purification.

Large unilamellar vesicles (LUVs) and phospholipid bicelles were used as membrane model systems. 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (POPG) were used to produce LUVs. 1,2-dihexanoyl-d_{22}-*sn*-glycero-3-phosphocholine (DHPC), 1,2-dimyristoyl-d_{54}-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-d_{54}-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (DMPG) were used to produce phospholipid bicelles. Phospholipids were obtained from Avanti Polar Lipids (Alabaster, AL, USA).

2.2. Preparation of samples containing LUVs for CD measurements

LUVs with a diameter of 100 nm were used for circular dichroism (CD) measurements because their large size and low curvature was deemed appropriate for studies of peptide–membrane interaction [28]. In order to produce neutral and acidic LUVs, POPC or a 4:1 mixture of POPC/POPG, respectively, were initially dissolved in chloroform to produce 20 mM stock solutions. The solutions were subsequently dried under a flow of nitrogen gas and stored under vacuum overnight to ensure that no solvent remained. These dried lipid films were soaked in 100 mM sodium phosphate buffer (pH 7.2) and vortexed for 10 min to obtain a more defined size distribution of the multilamellar vesicles. These solutions were then subjected to five freeze–thaw cycles to decrease lamellarity. Finally, to obtain uniform LUVs, the samples were extruded approximately 20 times through a polycarbonate microfilter with a pore size of 100 nm.

Peptide samples were prepared by dilution from the stock solutions of 20 mM LUVs, 1 mM AS1-, and 1 mM AS2-containing peptides to a final mixture containing 50 μM peptide, 50 mM sodium phosphate buffer (pH 7.2), and 1 mM neutral or acidic LUVs. This low concentration of LUVs was chosen to diminish disturbances due to light scattering. Samples containing a mixture of 25 μM AS1 and 25 μM AS2 peptides were also prepared for analysis. To study the nature of the interaction between the peptide and the LUVs, 150 mM KF was added to the samples [29]. KF was used because chloride salts are known to interfere with far-UV CD spectra [30].

2.3. Preparation of phospholipid bicelles for CD and NMR measurements

Small phospholipid bicelles with a q-ratio between 0.25 and 0.5 form isotropic solutions that are especially suitable for high-resolution membrane-interaction studies by NMR [31–35]. The q-ratio is the ratio of long-chained phospholipids [DMPC and DMPG] to short-chained phospholipids [DHPC].

Table 1
Properties of the AS1- and AS2-containing peptides used in the study.

Receptor[a]	GI[b]	Peptide	Sequence	Net charge	Hydrophobic moment (μH)[c]	ΔG_{wif} (kcal/mol)[d]
Af1503 (278–296)	1484730	AS1p–Af1503	STITRPIIELSNTADKIAE	−1	6.28	−0.71
$EnvZ_{Ec}$ (180–198)	947272	AS1p-$EnvZ_{Ec}$	RIQNRPLVDLEHAALQVGK	+1	3.84	−1.51
$NarX_{Ec}$ (177–195)	945788	AS1p-$NarX_{Ec}$	RLLQPWRQLLAMASAVSHR	+3	6.19	**−6.35**
Tar_{Ec} (214–232)	946399	AS1p-Tar_{Ec}	RMLLTPLAKIIAHIREIAG	+2	5.79	**−4.32**
Af1503 (310–328)	1484730	AS2p-Af1503	DEIGILAKSIERLRRSLKV	+2	5.42	−0.29
$EnvZ_{Ec}$ (211–229)	947272	AS2p-$EnvZ_{Ec}$	SEVRSVTRAFNHMAAGVKQ	+2	3.46	−1.51
$NarX_{Ec}$ (208–226)	945788	AS2p-$NarX_{Ec}$	EMAMLGTALNNMSAELAES	−3	5.57	−2.25
Tar_{Ec} (246–263)	946399	AS2p-Tar_{Ec}	EMGDLAQSVSHMQRSLTD	−2	4.81	−0.29

[a] The receptor that served as the source of AS1- and AS2-containing peptides. The position of the residues within the primary structure is provided in parentheses.
[b] The "GenInfo Identifier" sequence identification number.
[c] The hydrophobic moment (μH) of the peptides according to Eisenberg [58].
[d] The energy of interfacial partitioning (ΔG_{wif}) calculated within MPEx [59]. Significantly different values are indicated in bold font.