Antimicrobial Peptide from the Eusocial Bee *Halictus sexcinctus* Interacting with Model Membranes

Markéta Pazderková,^{1,2} Eva Kočišová,² Tomáš Pazderka,² Petr Maloň,¹ Vladimír Kopecký Jr.,² Lenka Monincová,¹ Václav Čerovský,¹ and Lucie Bednárová¹

¹*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo náměstí 2, 166 10 Prague 6, Czech Republic*

²*Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 5, 121 16 Prague 2, Czech Republic*

Correspondence should be addressed to Lucie Bednárová, bednarova@uochb.cas.cz

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Abstract. Halictine-1 (Hal-1)—a linear antibacterial dodecapeptide isolated from the venom of the eusocial bee *Halictus sexcinctus*—has been subjected to a detailed spectroscopic study including circular dichroism, fluorescence, and vibrational spectroscopy. We investigated Hal-1 ability to adopt an amphipathic α -helical structure upon interaction with model lipid-based bacterial membranes (phosphatidylcholine/phosphatidylglycerol-based large unilamellar vesicles and sodium dodecylsulfate micelles) and helix inducing components (trifluoroethanol). It was found that Hal-1 responds sensitively to the composition of the membrane model and to the peptide/lipid ratio. The amphipathic nature of the helical Hal-1 seems to favour flat charged surfaces of the model lipid particles over the nondirectional interaction with trifluoroethanol. Increasing fraction of polypyrrolone II type conformation was detected at low peptide/lipid ratios.

Keywords: Antibacterial peptides, halictine, micelle, liposome, phospholipid, circular dichroism, fluorescence, infrared spectroscopy

1. Introduction

There are many already known antimicrobial peptides (AMPs) with considerable therapeutic potential, but their exact mechanism of action still remains a matter of controversy [1]. AMPs interact with cytoplasmatic membrane, and their amphipathic structure plays an important role in this process. Simple models of membrane penetration involve formation of pores or dissolving membrane in a detergent-like manner [2, 3]. These processes lead to breakdown of the transmembrane potential causing leakage of cell content and finally the cell death. The mechanism of antibacterial action probably includes recognition and specific interaction with bacterial cell membranes inducing lipid clustering or lipid phase separation [4, 5].

We focus on changes of secondary structure of the small linear antimicrobial peptide Hal-1 (Gly-Met-Trp-Ser-Lys-Ile-Leu-Gly-His-Leu-Ile-Arg-NH₂) from the venom of the eusocial bee *Halictus sexcinctus* [6] as it responds to various membrane-mimicking environments. We employ circular dichroism (CD), fluorescence, and vibrational (IR and Raman) spectroscopy. Our model environments include 2,2,2-trifluoroethanol (TFE), sodium dodecyl sulfate (SDS), and large phospholipid liposomes.

2. Materials and Methods

The peptide Hal-1 was prepared by standard procedures of solid-phase peptide synthesis using Fmoc strategy. It was isolated as a trifluoroacetate salt, which in unbuffered aqueous solution exhibited pH ~6. The peptide concentration was 0.125 mg/mL for CD and fluorescence experiments and 10 mg/mL for FTIR. TFE was purchased from Merck and SDS from Sigma. 1,2-Dimyristoyl-*sn*-glycerol-3-phosphatidylcholine (PC) and 1,2-dimyristoyl-*sn*-glycerol-3-phospho-(1'-rac-glycerol) (sodium salt) (PG) were obtained from Avanti Polar Lipids. The phospholipid mixtures of various molar ratios were used for preparations of large unilamellar vesicles (LUVs) according to [7]. Size of LUVs (100 nm) was verified by dynamic light scattering (Zetasizer Nano, Malvern).

CD experiments were carried out using Jasco J-815 spectrometer in 0.1 cm quartz cells at room temperature. The final spectra were expressed as molar ellipticity Θ (deg·cm²·dmol⁻¹) per residue. Secondary structure content was estimated using Dichroweb software [8].

Fourier transform infrared (FTIR) spectra in the ATR mode were recorded (with resolution 2 cm⁻¹) on Bruker Vector 33 using the single reflection horizontal MIRacle ATR (Pike Technologies) with the diamond crystal and MCT detector. FTIR spectra in the transmission mode were recorded with spectral resolution 2 cm⁻¹ at room temperature on Bruker Equinox 55 using standard DTGS detector. The peptide solutions were measured in 6 μ m CaF₂ cell. The interfering water signal was subtracted using standard algorithm [9].

Steady-state fluorescence was measured on FluoroMax Z (Horiba Jobin Yvon) in a 10 mm quartz cell with excitation 280 nm in emission range 300–450 nm.

3. Results and Discussion

According to circular dichroism (Figure 1) Hal-1 in aqueous solution shows a prevailing truly unordered structure—a negative band at 198 nm as the sole significant feature (there is no positive long-wavelength band which is typical for polyproline II (PPII) conformation). There are still minor fractions of other conformations like α -helix (~10%) or some β -structure (not well shown by CD but clearly discernible in FTIR, see below). Hal-1 titrated with TFE shows gradually increasing content of α -helices (two negative minima at 205 and 222 nm) but only to a certain extent (~40% in 40% TFE solution). TFE does not seem capable of inducing a complete conformational change towards α -helix.

In SDS-containing environment CD spectra reveal even more complex behavior. The peptide is essentially nonhelical when SDS concentration is low. The presence of β -sheets and β -turns was confirmed by numerical analysis of CD data and by FTIR spectroscopy (see Table 1). α -Helical content reaches its maximum at SDS concentration around critical micelle concentration (cmc, 2–4 mM) while above cmc it decreases. With SDS concentration rising above cmc the spectrum again resembles the incompletely helical structure as induced by TFE. The observed spectral changes may be followed more clearly using difference CD spectra. With increasing concentration of SDS (mainly above cmc) we

Figure 1: CD spectra of Hal-1 titrated with (a) SDS and 50% TFE and (b) LUVs of different PC/PG compositions.

Table 1: Hal-1 secondary structure in the presence of SDS.

SDS (mM)	CD α -helix (%)	FTIR β -sheet (cm^{-1})	FTIR α -helix/unordered (cm^{-1})	FTIR β -turn (cm^{-1})	Fluorescence λ_{max} (nm)
0	12	1626 (m), 1640 (s), 1685 (w)	1654 (m)	1672 (w)	356
0.016	12	1626 (w), 1640 (m), 1684 (w)	1653 (m)	1672 (w)	—
0.16	13	1624 (w), 1687 (w)	1655 (m)	—	—
2	54	1624 (w), 1685 (w)	1656 (s)	—	331
4	60	1624 (w), 1685 (w)	1656 (s)	—	331
8	53	1624 (w), 1685 (w)	1655 (s)	—	331
16	53	1624 (w), 1685 (w)	1655 (s)	—	331

s: strong, m: medium; w: weak; λ_{max} : fluorescence maximum of the tryptophan band.

observe increasing portion of PPII conformation (strongly negative band below 200 nm accompanied by a positive band near 220 nm [10]).

To affirm this finding FTIR spectra were measured in analogous conditions in the presence of SDS (see Figure 2). In accordance with CD data, FTIR spectrum of Hal-1 in pure H_2O shows unordered structure with significant β -sheet (bands at 1626, 1640, and 1685 cm^{-1}) and β -turn (1672 cm^{-1}) fractions [11]. With increasing SDS concentration a formation of α -helical structure (1656 cm^{-1}) accompanied by a disappearance of β -sheet was also observed. In addition, a small shift of the band at 1656 cm^{-1} ascribed to α -helical or unordered structure [11] at higher than 4 mM concentrations of SDS was observed. This finding further hints at the possible formation of PPII conformations under such conditions.

Figure 2: FTIR amide I absorption and its second derivatives of Hal-1 titrated with (a) SDS and (b) LUVs of different PC/PG compositions.

Table 2: Hal-1 secondary structure in the presence of LUVs (L/P = 15).

LUV (PC/PG)	CD		FTIR		Fluorescence	
	α -helix (%)	β -sheet (cm ⁻¹)	α -helix/unordered (cm ⁻¹)	β -turn (cm ⁻¹)	$\lambda_{\max}$ (nm)	
0:0	12	1626 (m), 1640 (s), 1685 (w)	1654 (m)	1672 (w)	356	
1:0	18	1626 (m), 1640 (s), 1685 (w)	1657 (m)	—	360	
4:1	35	1626 (w), 1685 (w)	1656 (m)	—	—	
1:1	74	1626 (w), 1684 (w)	1655 (s)	—	—	
1:4	72	1626 (w), 1684 (w)	1654 (s)	—	331	

s: strong, m: medium; w: weak; $\lambda_{\max}$: fluorescence maximum of the tryptophan band.

As documented in Figure 2 and in Table 2 somewhat more sophisticated membrane models, based on PC-PG containing LUVs, affect Hal-1 conformation in a way that is under specific conditions (mutual ratios) quite comparable to SDS. To analyze data and extract the clear picture, it is in this case necessary to consider that the interaction of Hal-1 with LUVs is distinctly dependent on PC/PG ratio. The ability of Hal-1 to form α -helical structure is clearly enhanced by increasing proportion of PG in the lipid composition. At the ratio of PC/PG of about 4:1, LUVs act in a way quite similar to SDS. At first, α -helical structure prevails from the lipid/protein ratio (L/P) of ~ 8 till ~ 50 (α -helical fraction around 90%), but with larger L/P ratio (~ 200) it is again overshadowed by the PPII-type conformation. In

general, at the constant L/P ratio equal to 15 significant increase of α -helical fraction with increasing proportion of PG was observed. Nevertheless, α -helical structure was observed for LUVs containing PC only, but in the latter case $\sim 40\times$ higher L/P ratio was needed. These mainly CD-based results were supported by parallel FTIR measurements.

The wheel projection shows that Hal-1 in α -helical conformation adopts the amphipathic structure. Such an arrangement is more advantageous for the interaction with partners requiring access from one side (like flat surfaces) than with partners preferring access from all directions (like TFE, the known helix inducing agent). In the case that peptide is surrounded with low concentration of micelles, that is, concentration of SDS lower or around cmc, the peptide could easily keep the ideal amphipathic α -helical structure. However, if the micelles concentration increases, then several micelles can compete for interaction with a single peptide molecule and the amphipathic α -helical structure is no more an advantageous arrangement. Consequently, a gradual conformational change towards PPII left-handed helix was observed.

Fluorescence signal caused by tryptophan residue at the third position, which was observed at 356 nm in pure water and at 332 nm in SDS-containing environment, confirms that the peptide probably just adheres to the micelle surface with little penetration [12]. Probability of the peptide C-terminal embedding inside the micelle wall is low because of the presence of charged amino groups. A similar effect was observed in fluorescence when Hal-1 was surrounded by LUVs containing PG. It seems that the peptide reclines on the bilayer surface and it is not embedded into the bilayer. This could be a reason why drop-coating deposition Raman spectroscopy [13] showed no shifts in vibrations of the lipids upon the presence of the peptide. According to fluorescence spectra the interaction with liposomes takes place only if the peptide adopts α -helical structure (see Table 2).

4. Conclusions

The combined use of CD and vibrational spectroscopy provides detailed information about changes of Hal-1 secondary structure induced by interaction with model membranes. An increase of α -helical content accompanied by a decrease of random-coil and β -structures was observed as expected but additional formation of PPII structure appeared. This conformational change is caused by an increase of possible interactions of one peptide with several membrane surfaces. The amphipathic helical structure is no more the most favorable conformation, and the peptide should adopt a more advantageous arrangement. This is supported by fluorescence spectroscopy, which shows that the peptide is attached to the surface of the membrane with little penetration. To clarify the mode of action of chosen peptide and to further enlighten conformational aspects of its action additional more detailed analyses of Hal-1 and its analogs in interaction with model membranes should be yet carried out by different spectroscopic techniques.

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