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Spatial structure of heptapeptide Glu-Ile-Leu-Asn-His-Met-Lys, a fragment of the HIV enhancer prostatic acid phosphatase, in aqueous and SDS micelle solutions

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HIGHLIGHTS

- Central region of Prostatic Acid Phosphatase peptide was synthesized and characterized.
- Spatial structure of the peptide in water and in complex with sodium dodecyl sulfate was revealed.
- Complex formation was confirmed by ^{1}H NMR spectra.

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ABSTRACT

Prostatic acid phosphatase (PAP) is a protein abundantly present in human seminal fluid. PAP plays important role in fertilization. Its 39-amino-acid fragment, PAP(248–286), is effective in enhancing infectivity of HIV virus. In this work, we determined the spatial structure in aqueous solution of a heptapeptide within the PAP fragment, containing amino acid residues 266–272 (Glu-Ile-Leu-Asn-His-Met-Lys). We also report the structure of the complex formed by this heptapeptide with sodium dodecyl sulfate micelles, a model of a biological membrane, as determined by ^{1}H NMR spectroscopy and 2D NMR (TOCSY, HSQC-HECADE, NOESY) spectroscopy. Complex formation was confirmed by chemical shift alterations in the ^{1}H NMR spectra of the heptapeptide, as well as by the signs and values of NOE effects. We also present a comparison of the spatial structure of Glu-Ile-Leu-Asn-His-Met-Lys in water and in complex with sodium dodecyl sulfate.

1. Introduction

Prostatic acid phosphatase (PAP) is present in human seminal fluid and nearest tissue in large quantities [1]. PAP has been shown to have an important role in human normal physiological and pathological processes, such as fertilization, prostate carcinoma, and HIV infection [1–3]. A 39-amino-acid fragment within PAP, PAP(248–286), forms amyloid fibrils [1] that can greatly increase the risk of HIV infection, promoting virus attachment to the host cell [3]. These amyloid fibers, known as Semen-derived Enhancer of Viral Infection (SEVI), are thought to act as polycationic bridges, neutralizing the negative charge (the charge on the membrane surface) between the viral capsid and the host cell membrane [4]. However, the mechanism of action of PAP is still poorly understood.

The central region of PAP(248–286) is a heptapeptide, Glu-Ile-Leu-Asn-His-Met-Lys (EILNHMK, PAP(266–272)). Studying the interaction of the heptapeptide with the cellular membrane may be useful for understanding the mechanism of activity of PAP as a whole. The aim of this work was to determine the spatial structure of EILNHMK in complex with sodium dodecyl sulfate (SDS) micelles, which represent a cell surface membrane model. In this investigation, we have used high-resolution NMR spectroscopy, which is highly suitable for these studies, and several variations of this technique. Elucidating the spatial structure of the complex heptapeptide-SDS, as well as the structure of the heptapeptide in solution, may allow us to understand the mechanism(s) of PAP protein interaction with the cell surface. This work will lay the foundation for developing effective medications to inhibit the binding of HIV or other viral agents to the eukaryotic cell membrane.

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2. Experimental

Fmoc (9-fluorenylmethyloxycarbonyl groups)-protected amino acids of "peptide synthesis" grade were purchased from Applied Biosystems, Foster City, CA, USA. Peptide synthesis was made using 0.1 mmol automated fast Fmoc solid phase procedure using HBTU (O-Benzotriazole-N,N,N′,N′-tetramethyl-uronium-hexafluoro-phosphate) activation [5,6]. The procedure was performed on ABI 433A peptide synthesizer (Applied Biosystems, Foster City, CA, USA) at 293 K. Separation of the peptide substrate and the protecting groups was carried out in an acidic environment based on trifluoroacetic acid. The peptide was purified using high-performance liquid chromatography instrument Series 200 Perkin–Elmer HPLC System, Waltham, MA, USA. Vydac C18 column (Grace, IL, USA) was used at 328 K, water-acetonitrile linear gradient, flow rate 10 ml/min and backpressure 2800 psi. Concentration of acetonitrile in the water-acetonitrile mixture for the target peptide desorption was around 14%. The quality of the final product was characterized using MALDI-TOF (Matrix-Assisted Laser Desorption–Ionization) mass spectrometry. Purity of the peptide was estimated as better than 98%. The sample was stored at a temperature of −75 °C before usage.

Registration of 1D (^{1}H, ^{13}C) and 2D (^{1}H–^{1}H, ^{1}H–^{13}C) NMR spectra of the heptapeptide EILNHMK in aqueous solutions (H_2O + D_2O/95% + 5%) and lyotropic liquid crystalline medium was carried out on the NMR spectrometer (Bruker, AVANCE II-500) (500 MHz (^{1}H), 125.76 MHz (^{13}C)) at 288 K. The spectrometer operates in the mode of internal stabilization of the resonance line ^{2}H. ^{1}H NMR spectra were recorded using 90° pulses, a relaxation delay of 2 s and a spectral width of 8.6 ppm. For assignment of signals in the ^{1}H NMR spectra of the heptapeptide, 2D TOtal Correlation SpectroscopY (TOCSY) [7] was used. Samples studied were solutions of compounds (heptapeptide, or heptapeptide and SDS) in the indicated environments. For recording ^{1}H and ^{13}C NMR spectra, concentrations of substances were 0.5–0.6% by weight. Chemical shifts were measured relative to DSS (4,4-dimethyl-4-silapentan-1-sulfonic acid). The error in determining the values of dipole–dipole interaction did not exceed 1 Hz.

The residual value of the dipolar coupling (RDC) between the magnetic nuclei in EILNHMK was determined in a mixture of n-alkyl-poly(ethylene glycol) [$C_{12}E_5$, where 12 is the number of carbon n-alkyl atoms groups and 5 is the number of glycol groups in poly(ethylene glycol)]/n-hexanol and water [5.6% (w/w)]. The molar ratio (r) of n-alkyl-poly (ethylene glycol) to n-hexanol was 0.96. The lamellar L_α – phase state was confirmed by the quadrupole splitting of the deuterated water (D_2O) ^{2}H NMR signal [8]. The liquid-crystalline system was prepared based on n-alkyl-poly(ethylene glycol) and n-hexanol as described previously [9,10]. The SDS micelle solution in H_2O + D_2O was prepared at a concentration of 12 g/l. Concentration of peptide in heptapeptide–micelle mixture was 7 mg/ml. Heptapeptide powder was mixed with the micelle solution immediately before measurements were taken.

The ^{13}C CP/MAS (cross polarization and magic angle spinning of the sample) NMR spectrum (125.76 MHz) of the heptapeptide in the solid state (a crystalline powder sample) was recorded using a 4-mm MAS rotor at 288 K and a spinning rate of 7000 Hz and 10,000 Hz (200 ppm spectral width, 128 transients). The spectrum was referenced externally to a standard adamantane sample.

Nuclear Overhauser Effect SpectroscopY (NOESY) [7] ^{1}H–^{1}H 2D spectra were recorded in a phase-sensitive mode with 1024 points in the F2-direction and 256 points in the F1-direction with exponential filtration in both directions. Mixing time values, τ_m, were 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 and 0.40 s.

The experimental values of the residual dipole coupling were obtained by ^{1}H–^{13}C HSQC-HECADE (Heteronuclear Single Quantum Coherence spectroscopy) [11].

3. Results and discussion

3.1. NMR spectroscopy of heptapeptide EILNHMK in aqueous solution

The object of study (Fig. 1) is an oligopeptide comprising seven amino acid residues: glutamic acid, isoleucine, leucine, asparagine, histidine, methionine and lysine.

Fig. 2 shows the ^{1}H NMR spectrum (500 MHz) of the heptapeptide EILNHMK in an aqueous medium, and Table 1 shows the ^{1}H NMR chemical shifts of the heptapeptide.

Signals of the alpha proton, protons of methylene and methyl groups of the heptapeptide EILNHMK were assigned based on the data of two-dimensional ^{1}H–^{1}H TOCSY (Fig. 3, for example), data from the literature on the chemical shifts of protons in the amino acid fragments, and integrated intensities of the signals in the NMR spectra.

We determined the values of NMR chemical shifts of ^{13}C nuclei using data on the chemical shifts of protons. For this purpose, we generated the ^{1}H–^{13}C HSQC NMR spectrum of EILNHMK in aqueous solution. Signals from the carbon of the carboxyl group of amino acid fragments were determined on the basis of the HMBC (Heteronuclear Multiple Bond Correlation) spectra. Table 2 shows the chemical shifts of signals from the carbon groups of the heptapeptide.

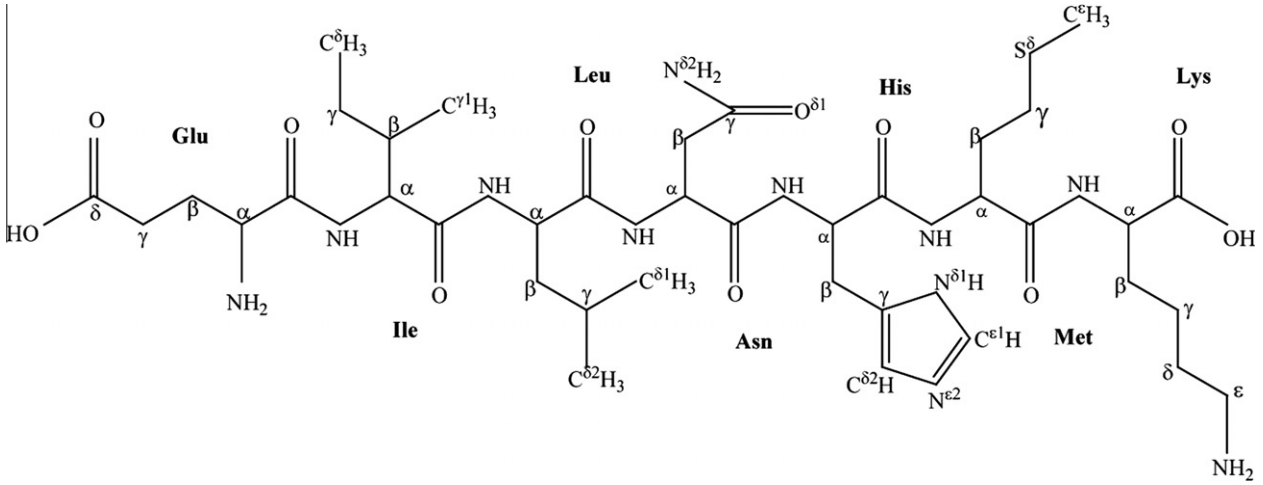

Fig. 1. Structural formula of the heptapeptide Glu-Ile-Leu-Asn-His-Met-Lys (EILNHMK).