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Interaction of anesthetic molecules with α-helix and polyproline II extended helix of long-chain poly-L-lysine

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ABSTRACT

The effect of halothane, enflurane, sevoflurane, and isoflurane molecules, as volatile anesthetics, on the α-helices and polyproline II extended helices (PPII) of long-chain poly-L-lysine (PLL) were studied using Fourier-transform infrared and vibrational circular dichroism spectroscopy. Uncharged and charged α-helices, as well as charged extended PPII helices, were subjected to anesthetic actions in solvents with different pD values or methanol to water ratios. A crucial factor responsible for hindering the anesthetic-PLL interactions is shown to be the ionization of amino groups of the PLL side chains. The α-helix to β-sheet transition was triggered only for the uncharged α-helical structures of PLL by the nonpolar anesthetics under study.

1. Introduction

Although volatile anesthetics have been used clinically to induce general anesthesia for over one hundred years (Buxton, 1912), a detailed understanding of the mechanisms by which these drugs induce their anesthetic effects still needs to be acquired. Little is known about the structural features of potential molecular targets of anesthetics. It is accepted that anesthetic binding sites exist on both protein and lipid molecules [1–6]. In the case of lipid-anesthetic systems, a nonspecific interaction dominates, while protein proponents favor a specific interaction. Anesthetic molecules, even if they are a heterogeneous group of small hydrophobic molecules, often discriminately interact with proteins. Anesthetic binding to proteins from rat neural membranes is affected by the type of protein conformation [7]. Additionally, in the case of calmodulin, anesthetics preferentially bind to certain protein conformations, which are characteristic of homo-calmodulin [8]. It is evident that the hydrophobicity, polarity, aromaticity and type of secondary structure influence the anesthetic binding to protein molecules [9–11].

To further increase the knowledge on the character of the interaction of anesthetic molecules with particular protein secondary structures, the long-chain poly-L-lysine (PLL) is studied in the presence of four volatile anesthetics, that is, halothane, enflurane, sevoflurane and isoflurane, using Fourier-transform infrared (FTIR) and vibrational circular dichroism (VCD) spectroscopy. Both of these experimental methods are commonly used because they provide valuable and detailed structural information not only about the protein structure but also for many other biomolecules, such as lipids, which assemble into bilayer structures [12–16]. Since PLL molecules assume well defined α-helices, β-sheets and polyproline II extended helices (PPII), which can easily be changed by simple variations in the external conditions, this peptide serves as an excellent model for the structures of natural proteins [13,17–19]. By variations in the pH values of the aqueous solvents and in the methanol to water ratio, α-helix and PPII structures of PLL, which differ in the charge of the amino acid side chains, can be exposed to the anesthetic actions. Halogenated ethers or alkanes are some of the most frequently used inhalational anesthetic agents for different surgical operations. In modern anesthesia, the most frequently used anesthetics in clinical practice contain sevoflurane [20]. Isoflurane, enflurane or halothane molecules are also in clinical use [20]. Moreover, some of these volatile anesthetics can induce myocardial protection [21]. In this paper, enflurane, sevoflurane and isoflurane were chosen as halogenated ethers with effective inhalation anesthetic properties. Halothane was used as an example of halogenated alkane anesthetic molecules, and its effect on the secondary structure of PLL was compared with that of the halogenated ether molecules.

2. Material and Methods

Heavy poly-L-lysine hydrobromide (250 kDa) was purchased from Sigma Aldrich (Seelze, Germany). Enflurane (97% purity) was obtained

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from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), isoflurane (99% purity) was obtained from Apollo Scientific Ltd. (Manchester, UK), halothane (99% purity) was obtained from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), and sevoflurane (>98% purity) was obtained from Carbosynth Ltd. (Berkshire, UK). All of these compounds were used without further purification. The final concentration of PLL dissolved in D_2O at pD 11.1 at the temperature of 10 °C was 40 mg/mL, and for PLL dissolved in 50/50% or 90/10% (v/v) methanol/water mixtures was 50 mg/mL. The final concentration of anesthetics in PLL containing samples was 8 mM. The pD value was adjusted with concentrated NaOD using a model FiveEasy pH-meter (Mettler Toledo) with a correction of 0.4. To investigate the effect of anesthetic molecules on two different secondary structures of PLL, solvents with different chemical composition, pD and temperature were used. According to the literature data [13,17–19], by simple changes in these external conditions, PLL can adopt α-helical or PPII structures. The formation of α-helical structures of PLL was triggered in two different ways: (1) by dissolving PLL in a pure water solution at pD 11.1 at a temperature of 10 °C, or (2) by dissolving PLL in a methanol/water mixture with a 90/10% (v/v) ratio at room temperature. The formation of PPII structures of PLL was induced by dissolving PLL in pure water with pD 7 or in a methanol/water mixture with a 50/50% (v/v) ratio at room temperature.

To study the transition of α-helical structures into β-sheet forms triggered by the presence of anesthetic molecules, we decided to use the long-chain PLL, which has a high propensity to form β-sheets [13,22]. Decreasing the chain length of PLL clearly decreases the ability of this polypeptide to transform α-helices to β-sheets with increasing temperature, and this process is even completely inhibited for the shorter lengths [22]. Thus, using the long-chain PLL with a high capacity of β-sheet formation allowed us to study the α-helix to β-sheet transition in alkaline aqueous solution triggered by the presence of anesthetic molecules even at a low temperature, which generally does not favor this process. Another advantage of using long-chain PLL is that this polypeptide in methanol-rich water solvents predominantly forms α-helical structures [17,23], but with a higher positive charge than that for α-helices formed in pure water in alkaline conditions. By decreasing the PLL chain length, the content of α-helices in methanol-rich water solvents decreases, and finally, for the shortest PLL molecules in such conditions, the secondary structure is represented mainly by random coil forms [23].

As the pKa value of the amine group in the side chain of Lys is 10.5, PLL dissolved in an aqueous solution with pD 11.1 is in an uncharged form, but is in a charged state at pD 7. The presence of charged α-helical forms of PLL in methanol-rich water solvents was proved by a reduction of the sedimentation rate, which was characteristic for charged polymers [23]. The detailed characterization of the ionization state of PLL was described by the titration curve of PLL in a 95% methanol mixture with water [24].

2.1. FTIR and VCD Studies

A Nicolet iS50 FT-IR spectrometer (Thermo Scientific) extended for VCD measurements and equipped with an MCT liquid nitrogen cooled detector was applied to obtain the FTIR and VCD spectra of pure and anesthetic-mixed PLL molecules dissolved in either alkaline aqueous solvent or methanol/water mixtures. A CaF_2 cell with a 56-μm Teflon spacer was used. 128 Scans with a resolution of 2 cm^{-1} and 15,000 scans with a resolution of 8 cm^{-1} were collected for each FTIR and VCD spectrum, respectively. The pretreatment of the raw spectra was as follows: a subtraction of the water spectrum from the peptide spectrum; a baseline correction with a linear function; and a normalization to a constant total area in the analyzed regions. The GRAMS/32 AI software version 8 (Galactic Industries Corporation, Thermo Scientific, Poland) was used for the analysis.

3. Results

3.1. Effect of Anesthetic Molecules on α-Helix Structures of PLL

In alkaline pH and at a low temperature, the secondary structure of the long-chain PLL under study was represented mainly by α-helical conformations. The amide I′ band of PLL in D_2O solution at pD 11.1 is presented in Fig. 1A and B. At the temperature of 10 °C, this band was composed of at least three subbands, centered at 1668, 1638 and 1622 cm^{-1}, with different contributions. The most dominant subband had a maximum at 1638 cm^{-1}, characteristic of the α-helical structures of PLL [13,18,19] (Fig. 1B). The assignment of bands with the maxima at 1668 and 1622 cm^{-1} is still under discussion [13,22,25]. The low-frequency band may originate from the end fragments of α-helices, which are disordered and more solvated, and the second, high-frequency band can be assigned to the turns and disordered forms of PLL under study. Changes in a shape of the amide I′ band of PLL in the presence of four volatile anesthetics, represented by halothane, enflurane, sevoflurane, and isoflurane molecules, are shown in Fig. 1A. The anesthetics under study caused an increase in the intensity of a new band at 1610 cm^{-1} and also in a much less intense band centered at

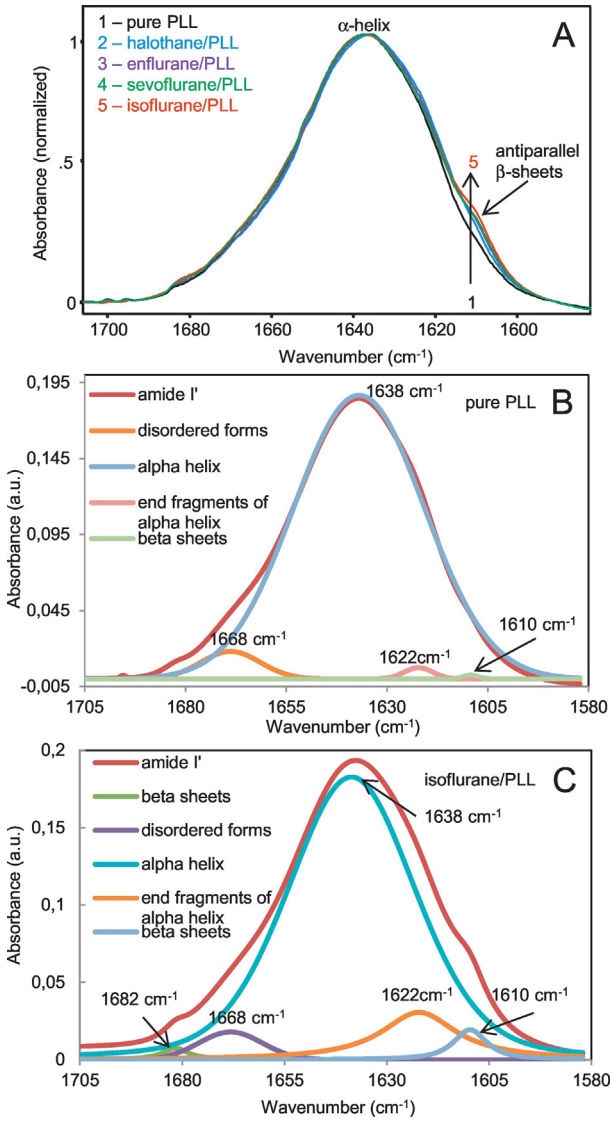

Fig. 1. (A) The amide I′ bands of pure and anesthetic-mixed long-chain PLL dissolved in water with pD 11.1, at the temperature of 10 °C. Peak fitting with Gaussian functions of amide I′ bands of the pure PLL (B) and mixed with isoflurane molecules (C).