International Congress Series 1283 (2005) 155–159

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# Structural changes in a four-alpha-helix bundle protein following sevoflurane binding

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**Abstract.** A molecular understanding of volatile general anesthetic mechanisms of action will ultimately require high-resolution structural descriptions of anesthetic–protein complexes. Structural changes in proteins following anesthetic binding have been technically difficult to detect, but presumably underlie many of the resulting reversible alterations in protein function. Using three different spectroscopic approaches, evidence is presented that binding of a modern general anesthetic to the four-α-helix bundle $(A\alpha_2\text{-L1M/L38M})_2$ results in conformational changes in the target. Comparable structural changes in in vivo central nervous system protein targets may underlie some, or all, of the behavioral effects of these widely used clinical agents. © 2005 Elsevier B.V. All rights reserved.

*Keywords:* Fluorescence spectroscopy; Circular dichroism spectroscopy; Nuclear magnetic resonance spectroscopy; Four-α-helix bundle; Anesthetic–protein interaction

## 1. Introduction

How a bound volatile general anesthetic reversibly alters protein function remains unknown to this date. Perturbation of protein structure following anesthetic binding is an attractive option but this has been difficult to detect experimentally because the changes

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doi:10.1016/j.ics.2005.06.099

are likely to be quite subtle, involving tertiary and/or quaternary structure. The only X-ray crystal structure of a complex between a modern volatile general anesthetic and a protein currently available is that of halothane and human serum albumin [1], which uncovered no structural changes following anesthetic binding. In addition, anesthetic-enhanced sodium transport activity by the lipid-soluble antimicrobial peptide gramicidin A is not associated with any structural change as assessed by high-resolution nuclear magnetic resonance spectroscopy [2]. Using three different spectroscopic approaches, evidence is presented that binding of a modern general anesthetic to the four-α-helix bundle $(A\alpha_2\text{-L1M/L38M})_2$ results in structural changes. The transmembrane domains of membrane proteins typically consist of bundles of α-helices [3–5]. The ability of four-α-helix bundles to model the transmembrane domains is therefore being examined in order to provide detailed descriptions of anesthetic–protein interactions with the ultimate goal of understanding the mechanisms of anesthetic action. Comparable structural changes in in vivo protein targets such as the Cys-loop ligand-gated ion channel γ-aminobutyric acid type A receptor and the *N*-methyl-D-aspartate receptor may underlie some, or all, of the behavioral effects of these widely used clinical agents.

## 2. Materials and methods

Sevoflurane (fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl) ethyl ether) was obtained from Abbott Laboratories (North Chicago, IL). All other chemicals were of reagent grade.

The peptide $A\alpha_2$-L1M/L38M was expressed and purified as described [6]. Laser desorption mass spectrometry confirmed the peptide identity. The expected molecular weight was 6863.1 Da, and the experimental value was 6863.0 Da.

Binding of sevoflurane to the four-α-helix bundle $(A\alpha_2\text{-L1M/L38M})_2$ was determined using steady-state intrinsic tryptophan fluorescence measurements [7] on an RF-5301PC spectrofluorometer (Shimadzu, Columbia, MD). Tryptophan was excited at 280 nm (bandwidth 1.5 nm) and emission spectra (bandwidth 3 nm) recorded with the control peak at 324 nm. The quartz cell had a path length of 10 mm and a Teflon stopper. The temperature of the cell holder was controlled at 25.0 ± 0.1 °C. The buffer was 130 mM NaCl, 20 mM sodium phosphate, pH 7.0.

One-dimensional proton nuclear magnetic resonance spectra of the aromatic-amide fingerprint region were recorded on a Varian Inova 500 MHz instrument with a 90° pulse-acquisition sequence [8] (spectral width of 10 kHz, water presaturated for 1.5 s, 128 scans). The $A\alpha_2$-L1M/L38M concentration was 590 μM in 20 mM phosphate buffer (containing 10% $D_2O$), pH 7.0. Sevoflurane (50 μl, final concentration 540 μM) was added from a stock solution of 8.0 mM to the NMR tube containing $(A\alpha_2\text{-L1M/L38M})_2$ to yield approximately a 1:1 concentration, resulting in a final $A\alpha_2$-L1M/L38M concentration of 550 μM. The spectra were processed using Felix 2.3 from MSI (San Diego, CA) on a Silicon Graphics (Mountain View, CA) workstation. The spectra were referenced with respect to the water peak (4.81 ppm at 20 °C).

$^{15}N$–$^{1}H$ heteronuclear single quantum coherence (HSQC) NMR spectra were recorded at 11.7 T at 25 °C and pH 4.7 on a Varian Inova 500 MHz instrument equipped with a cryoprobe using standard methods [9]. The spectra were processed using NMRPipe [10]

on a personal computer running the Linux operating system. The HSQC NMR spectra were referenced with respect to the water peak (4.78 ppm at 25 °C).

### 3. Results

Sevoflurane bound to the hydrophobic core of the four-α-helix bundle $(A\alpha_2\text{-L1M/L38M})_2$ with a $K_d = 280 \pm 10$ μM, as assessed by W15 fluorescence spectroscopy, in agreement with the clinical $EC_{50}$ value in man of 260 μM [11]. Fig. 1 shows that the binding of sevoflurane to the hydrophobic core of the four-α-helix bundle $(A\alpha_2\text{-L1M/L38M})_2$ is accompanied by a progressive red shift on the order of 5 nm in the W15 fluorescence emission maximum. Because sevoflurane does not quench tryptophan fluorescence directly, this decrease in the W15 quantum yield and the accompanying red shift in the fluorescence emission maximum wavelength provide evidence that binding of the anesthetic changes the environment of the W15 residue. The 5-nm red shift in the W15 fluorescence emission maximum indicates that the indole ring is transferred into a somewhat more polar environment in the interior of the four-α-helix bundle $(A\alpha_2\text{-L1M/L38M})_2$ upon anesthetic binding [12]. The most likely scenario is that the W15 side-chain orients into closer proximity to a backbone carbonyl group, which would simultaneously quench the fluorescence [13] and also provide a higher effective local dielectric constant, allowing for the progressive 5-nm red shift in the emission maximum. Backbone carbonyl groups represent common polar moieties in the predominantly hydrophobic interior of proteins.

Near-ultraviolet circular dichroism spectroscopy revealed that the binding of sevoflurane to the hydrophobic core of $(A\alpha_2\text{-L1M/L38M})_2$ altered the orientation of the three aromatic residues (W15, F12, and F52) of the four-α-helix bundle protein [6]. Below 270 nm, the CD signal changes its sign from positive to negative upon sevoflurane binding. Above 270 nm, in the absence of anesthetic, the protein has positive CD signals with maximum intensities at 287 and 295 nm. After sevoflurane binds, the CD signal becomes negative throughout the tryptophan wavelength range.

One-dimensional proton nuclear magnetic resonance spectroscopy demonstrated that bound sevoflurane leads to a more structured conformation being assumed by the four-α-helix bundle

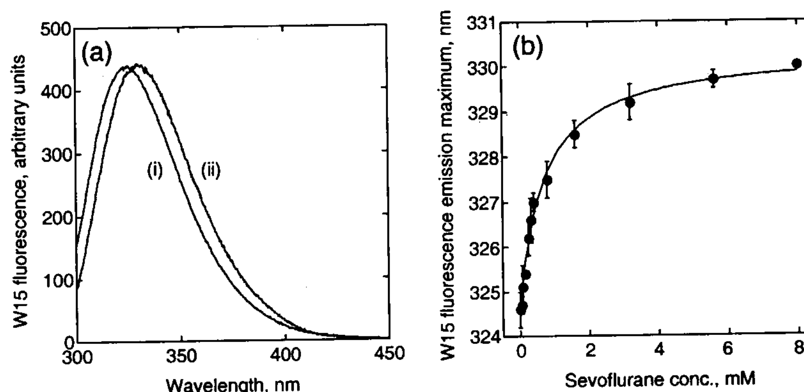


Fig. 1. (a) The W15 emission spectra in the absence of sevoflurane (i) and in the presence of 8 mM sevoflurane (ii). The (ii) spectrum (in the presence of sevoflurane) has been multiplied by a factor of 2.436 in order to make it comparable in intensity to the control spectrum (i). (b) The progressive red shift in the W15 emission maximum upon the addition of increasing concentrations of sevoflurane. Data points in (b) are the means of three separate experiments with the error bars representing the standard deviations.

protein, because the peaks in the chemical shift range of 10.5–6.0 ppm (aromatic and amide region) become sharper. The $^{15}N$–$^{1}H$ HSQC spectra of the four-α-helix bundle $(A\alpha_2\text{-L1M/L38M})_2$ at 25 °C indicate that at least 15 of the 62 backbone amide cross-peaks present in the free protein undergo chemical shift changes in the presence of sevoflurane. Such widespread structural changes upon anesthetic binding are predicted to translate into alterations in normal protein function. These observations indicate that a complex is being formed, in line with the fluorescence and circular dichroism data, and that the binding of the anesthetic induces structural changes in the four-α-helix bundle.

## 4. Discussion

A molecular understanding of volatile anesthetic mechanisms of action will ultimately require high-resolution structural descriptions of anesthetic–protein complexes. Structural changes in proteins following anesthetic binding have been technically difficult to detect, but presumably underlie many of the resulting reversible alterations in protein function. Using three different spectroscopic approaches, evidence is presented that binding of a modern general anesthetic to the four-α-helix bundle $(A\alpha_2\text{-L1M/L38M})_2$ results in conformational changes in the target. Comparable structural changes in currently favored in vivo central nervous system protein targets such as the Cys-loop ligand-gated ion channel γ-aminobutyric acid type A receptor and the *N*-methyl-D-aspartate receptor may underlie some, or all, of the behavioral effects of these widely used clinical agents. Ongoing X-ray crystallographic studies on the four-α-helix bundle $(A\alpha_2\text{-L38M})_2$ in the absence and presence of bound volatile general anesthetics should provide more detailed insight into these structural perturbations.

## Acknowledgments

This work was supported by National Institutes of Health Grant GM55876. Mass spectrometry was performed at the Protein Chemistry Laboratory, University of Pennsylvania, Philadelphia, PA, USA.

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