Binding of the Volatile Anesthetic Chloroform to Albumin Demonstrated Using Tryptophan Fluorescence Quenching*

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The site(s) of action of the volatile general anesthetics remain(s) controversial, but evidence in favor of specific protein targets is accumulating. The techniques to measure directly volatile anesthetic binding to proteins are still under development. Further experience with the intrinsic protein fluorescence quenching approach to monitor anesthetic-protein complexation is reported using chloroform. Chloroform quenches the steady-state tryptophan fluorescence of bovine serum albumin (BSA) in a concentration-dependent, saturable manner with a \( K_d = 2.7 \pm 0.2 \) mM. Tryptophan fluorescence lifetime analysis reveals that the majority of the quenching is due to a static mechanism, indicative of anesthetic binding. The ability of chloroform to quench BSA tryptophan fluorescence was decreased markedly in the presence of 50% 2,2,2-trifluoroethanol, which causes loss of tertiary structural contacts in BSA, indicating that protein conformation is crucial for anesthetic binding. Circular dichroism spectroscopy revealed no measurable effect of chloroform on the secondary structure of BSA. The results suggest that chloroform binds to subdomains IB and IIA in BSA, each of which contains a single tryptophan. Earlier work has shown that these sites are also occupied by halothane. The present study therefore provides experimental support for the theory that structurally distinct general anesthetics may occupy the same domains on protein targets.

The site(s) of action of the volatile general anesthetics remain(s) to be defined. Current consensus favors membrane proteins in the central nervous system which function as ion channels and/or neurotransmitter receptors (1, 2). In support of this view are the numerous studies demonstrating changes in channels and/or neurotransmitter receptors (1, 2). In support of proteins in the central nervous system which function as ion main(s) to be defined. Current consensus favors membrane proteins. Using these approaches it has been possible to demonstrate that discrete binding sites for volatile general anesthetics exist on water-soluble proteins. Recent results for photoaffinity labeling of the Torpedo nicotinic acetylcholine receptor with halothane suggest that such sites are also present on membrane proteins (13).

A long held assumption in the anesthetic mechanisms field has been that a single site exists in the central nervous system which is the relevant site of action of the structurally diverse general anesthetics (14). No direct binding studies have addressed this issue. Indirect evidence has been obtained using both functional and competition binding studies on model systems. Thus, Franks and Lieb (15) showed that a number of anesthetics, ranging from halogenated alkanes and ethers to \( n \)-alkanes, alter the activity of firefly luciferase, apparently by competing for the same site on the enzyme which normally binds the substrate luciferin. In addition, competition studies on anesthetic binding to serum albumin using \(^{19}F\) NMR spectroscopy (7), photoaffinity labeling (9), and fluorescence spectroscopy (11) suggest that different volatile anesthetics may bind to the same site(s) on certain proteins. However, competitive binding displayed by different ligands may result from allosteric effects, whereby binding of ligand A at a certain site causes a conformational change in the protein such that binding of ligand B at a separate site is altered (16). In this study, the observations on halothane binding to proteins as monitored by intrinsic tryptophan fluorescence quenching are extended to include the volatile general anesthetic chloroform. The approach used allows direct monitoring of anesthetic binding to the protein and, in addition, provides information about the location of the anesthetic in the protein matrix. The results indicate that chloroform occupies the same binding site on this model mammalian protein as does halothane.

**EXPERIMENTAL PROCEDURES**

*Materials—* Chloroform (high performance liquid chromatography grade) was obtained from Fisher Scientific. Fatty acid-free bovine serum albumin (BSA), fatty acid-free human serum albumin (HSA), myoglobin (from horse skeletal muscle), and tryptophan were purchased from Sigma. Hexane and 2,2,2-trifluoroethanol (NMR grade) were from Aldrich. All other chemicals were reagent grade.

The buffer used for all fluorescence experimentation was 130 mM sodium chloride, 20 mM sodium phosphate, pH 7.0. Proteins were equilibrated into a desensitized conformation (3), and halothane inhibits membrane protein function in the presence of anesthetics. For this view are the numerous studies demonstrating changes in channels and/or neurotransmitter receptors (1, 2). In support of proteins in the central nervous system which function as ion main(s) to be defined. Current consensus favors membrane proteins. Using these approaches it has been possible to demonstrate that discrete binding sites for volatile general anesthetics exist on water-soluble proteins. Recent results for photoaffinity labeling of the Torpedo nicotinic acetylcholine receptor with halothane suggest that such sites are also present on membrane proteins (13). A long held assumption in the anesthetic mechanisms field has been that a single site exists in the central nervous system which is the relevant site of action of the structurally diverse general anesthetics (14). No direct binding studies have addressed this issue. Indirect evidence has been obtained using both functional and competition binding studies on model systems. Thus, Franks and Lieb (15) showed that a number of anesthetics, ranging from halogenated alkanes and ethers to \( n \)-alkanes, alter the activity of firefly luciferase, apparently by competing for the same site on the enzyme which normally binds the substrate luciferin. In addition, competition studies on anesthetic binding to serum albumin using \(^{19}F\) NMR spectroscopy (7), photoaffinity labeling (9), and fluorescence spectroscopy (11) suggest that different volatile anesthetics may bind to the same site(s) on certain proteins. However, competitive binding displayed by different ligands may result from allosteric effects, whereby binding of ligand A at a certain site causes a conformational change in the protein such that binding of ligand B at a separate site is altered (16). In this study, the observations on halothane binding to proteins as monitored by intrinsic tryptophan fluorescence quenching are extended to include the volatile general anesthetic chloroform. The approach used allows direct monitoring of anesthetic binding to the protein and, in addition, provides information about the location of the anesthetic in the protein matrix. The results indicate that chloroform occupies the same binding site on this model mammalian protein as does halothane.
Chloroform Binding to Albumin Using Fluorescence Spectroscopy

Irradiated with chloroform (final concentration 35 mM) in gas-tight Hamilton syringes. Chloroform-equilibrated protein was diluted with predetermined volumes of plain protein (treated in the same manner) to achieve the final anesthetic concentrations shown in the figures.

BSA and HSA were used without further purification (purity > 96% by electrophoresis). There are two tryptophans in BSA, at positions 134 and 212. Trp-134 is thought to be more solvent-exposed than is Trp-212 (17). HSA has a single Trp-214. Protein concentrations were determined as described (11).

Steady-state Fluorescence Measurements—Steady-state fluorescence measurements were performed with a spectrophotometer F-4500 (Hitachi, Danbury, CT). Tryptophan was excited at 295 nm and emission spectra recorded with peaks at 344 nm for BSA and 342 nm for HSA at pH 7.0. This excitation wavelength minimizes radiationless energy transfer from tyrosine residues. A 10-mm path length quartz cell with a Teflon stopper was used. Care was taken to avoid the presence of significant air pockets. The cuvette was placed in a thermostatically controlled cell holder with a temperature of 25.0 ± 0.1 °C. Both excitation and emission slit widths were 5 nm. Chloroform was determined to have an extinction coefficient at 200 nm of 150 M−1 cm−1 (data not shown), so inner filter effects were negligible.

Steady-state fluorescence intensity data were fitted to the equation

\[ F = 1 - (Q_{\text{max}} + [\text{chloroform}]K_d + [\text{chloroform}]) \]  

(Eq. 1)

where \( F \) is fluorescence intensity, \( Q_{\text{max}} \) is the maximum fluorescence that can be quenched, and \( K_d \) is the dissociation constant for the binding of anesthetic to albumin, as described previously (11).

Fluorescence Lifetime Data Acquisition and Analysis—Fluorescence decay kinetics were measured on a K2 multifrequency stopped-flow, phase and modulation spectrofluorometer (ISS Inc., Champaign, IL). Albumin solutions in Teflon-stoppered 5-mm path length quartz cells, with varying concentrations of chloroform, were excited at 295 nm with a 4-nm bandwidth, at room temperature. Emitted photons passed through a 308-nm cut-on filter, followed by a polarizer set at the magic angle (55°) to the vertical to minimize the effect of rotational diffusion. A glycogen solution served as the scattered excitation light reference. Fluorescence lifetime data were obtained and analyzed at 20 modulation frequencies (1–300 MHz) using 0.2° and 0.004° standard limits for phase and modulation, respectively. ISS software was used for both the acquisition (ISSL) and analysis (ISISS187) of the fluorescence decay data. The fluorescence decay intensity at time \( t \), \( I(t) \), was analyzed as a sum of exponentials: \( I(t) = \sum a_i \exp(-t/\tau_i) \), where \( \tau_i \) is the lifetime and \( a_i \) is the fractional intensity of the \( i \)-th decay component (18, 19). Global \( \chi^2 \) minimization was used as a criterion for the goodness of fit of the applied model to the experimental values. A reduced \( \chi^2 \) value less than 1.3 was regarded as an acceptable description of the data (20, 21). The fluorescence lifetime standard \( \tau \)-terphenyl in ethanol was used to evaluate instrument performance, yielding a \( \tau = 1.00 \pm 0.000 \) ns (\( n = 4 \) in good agreement with the accepted value (18).

Circular Dichroism Spectroscopy—CD spectra were recorded on a model 62 DS Spectropolarimeter (Aviv, Lakewood, NJ). BSA (1.0 μM) in 10 mM potassium phosphate buffer at pH 7.0 was equilibrated with chloroform in gas-tight Hamilton syringes. A 1-mm path length quartz cell with a Teflon stopper was used. The cell holder was temperature-controlled at 25.0 ± 0.1 °C. The bandwidth was 1.00 nm, with a scan step of 0.5 nm and an average scan time of 3.0 s.

Gas Chromatography—Buffer concentrations of chloroform were determined using gas chromatography on an HP 6890 series instrument (Hewlett Packard, Wilmington, DE). Aliquots (100 μl) of chloroform-equilibrated protein solutions were added to 2.0 ml of hexane in 5.0 ml glass tubes with Teflon stoppers. Samples (3 μl) of chloroform in hexane were injected into a 30-m 5% phenyl-substituted methylpolysiloxane column (internal diameter of 0.32 mm), using nitrogen carrier gas at a flow rate of 188 ml/min. The oven temperature was 80 °C, with the electron capture detector operating at 350 °C.

Curve Fitting and Statistics—Best fit curves were generated with the Kaleidograph (Abelbeck Software, Reading, PA) program. Data are expressed as means ± S.D. Data points are the averages of at least three experiments conducted with separate samples.

RESULTS

Fig. 1 shows fluorescence emission spectra for BSA in the presence of increasing concentrations of chloroform. Chloroform causes a decrease in the protein tryptophan fluorescence quantum yield. A slight blue shift (2 nm at 10.5 mM chloroform) in the emission wavelength maximum (344 nm for uncleched protein) was observed, suggesting that binding of the anesthetic is associated with changes in the dielectric environment of at least one of the two indole rings in BSA. Fig. 2a shows that coequilibration of chloroform with BSA at pH 7.0 causes a concentration-dependent decrease in the intrinsic tryptophan fluorescence. The line through the data points is a best fit curve derived using Equation 1. The \( K_d = 2.7 ± 0.2 \) mM, with a \( Q_{\text{max}} = 1.01 ± 0.02 \), implying that the fluorescence of both tryptophan residues in BSA is effectively quenched. The free energy of this interaction (\( \Delta G^f = RT \ln K_d \), molar standard state) at 25 °C is −3.5 ± 0.1 kcal/mol, or equivalent to up to three conventional hydrogen bonds (22, 23).

To evaluate the importance of the native BSA conformation to chloroform binding, experiments were performed in 50% 2,2,2-trifluoroethanol. Trifluoroethanol negates the hydrophobic interactions that are in part responsible for the native folded state of proteins (24) while maintaining secondary structure (25, 26). Fig. 2b shows that there is a significant decrease in the amount of quenching of BSA tryptophan fluorescence in the presence of 2,2,2-trifluoroethanol compared with that at pH 7.0 (Fig. 2a). The diminished quenching of partially unfolded BSA tryptophan fluorescence suggests that tertiary structural features are crucial for creating suitable binding sites for chloroform on a protein target.

The effect of chloroform on free L-tryptophan fluorescence at pH 7.0 was assessed (Fig. 2c). A linear decrease in tryptophan fluorescence was observed with a \( Q_{\text{max}} = 33 ± 1 % (n = 3) \) at 35 mM chloroform. The slope of this fluorescence quenching corresponds to a \( K_{\text{sv}} = 10.3 ± 0.4 \) M−1 (see Fig. 4).

Also shown in Fig. 2d is the effect of chloroform on myoglobin tryptophan fluorescence at pH 7.0. Myoglobin has two tryptophan residues (27), at positions 7 and 14, one of which is buried, and the other of which is exposed to the solvent (28). Addition of chloroform causes a linear decrease in the intrinsic myoglobin tryptophan fluorescence with lack of saturation over the concentration range studied (up to 35 mM chloroform). At 35 mM chloroform 22 ± 4% (n = 3) of the total tryptophan fluorescence of myoglobin is quenched. This degree of myoglobin fluorescence quenching is somewhat less than that observed with free l-tryptophan, indicating that collisional encounters between chloroform and the protein are the principal mechanism responsible, as opposed to a binding interaction.

Fig. 2a shows that chloroform is able to quench the fluorescence of both of the tryptophan residues in BSA. Trp-134 and Trp-212 are located in subdomains IB and IIA, respectively, of BSA (17), indicating the presence of at least two chloroform binding sites. To measure the relative affinities of the two...
chloroform binding sites identified by tryptophan fluorescence quenching in BSA, experiments were carried out with HSA, which contains a single tryptophan at the conserved position 214 (17). Trp-214 in HSA is analogous to Trp-212 in BSA (17). Fig. 3 shows the concentration-dependent quenching of HSA and BSA tryptophan fluorescence by added chloroform. Using Equation 1, the $K_d$ for HSA was 5.6 ± 0.3 mM, and the $Q_{max}$ for HSA (compared with the $K_d$ of 2.7 ± 0.2 mM for BSA).

Assuming that the chloroform binding site in the vicinity of the Trp-212 site in BSA is similar to that in HSA, one can calculate the affinity of the more solvent-exposed Trp-134 site for chloroform in BSA. The $K_d$ for the Trp-134 site can be taken as 5.6 ± 0.3 mM and the data in Fig. 2a fitted to an equation of the form

$$F = 1 - ([0.37 + [\text{chloroform}] / (5.6 + [\text{chloroform}])]  
+ (0.63 + [\text{chloroform}] / (K_d^{134} + [\text{chloroform}]) \quad \text{(Eq. 2)}$$

where chloroform concentrations are expressed in millimolar units; 0.63 and 0.37 are the experimentally determined contributions (17) of each of the two tryptophan residues to the total fluorescence at 344 nm in BSA; and $K_d^{134}$ is the dissociation constant of the Trp-134 site. Equation 2 allows a value of 1.5 ± 0.1 mM to be calculated for the $K_d^{134}$ of the more solvent-exposed site. Almost identical $\chi^2$ (0.00687 versus 0.00732 for one versus two affinity models, respectively) and correlation coefficients (both 0.998) are obtained with both solutions.

Because electron transfer (29, 30) from the excited indole ring to chloroform might be responsible for the observed fluorescence quenching, which would involve free radical production, the reversibility of the protein-anesthetic interaction was examined. Samples of BSA with chloroform (35 mM) were examined fluorometrically and degassed with nitrogen for 45 min, at which time a second tryptophan emission spectrum was obtained. Compared with a control sample of BSA treated in the same manner, the BSA exposed to chloroform and to ultraviolet light, and subsequently degassed, retained 86 ± 4% ($n = 5$) of its tryptophan fluorescence. This result indicates that the vast majority of the observed fluorescence quenching is reversible. Furthermore, the reversibility means that the observed quenching of the tryptophan fluorescence is not caused by chloroform-induced aggregation, or precipitation, of BSA.

As shown in Fig. 2c, chloroform has a less pronounced quenching effect on free t-tryptophan fluorescence in buffer at pH 7.0 compared with its effect on albumin fluorescence. Experiments were performed in methanol to examine the effect of higher concentrations of chloroform (to 170 mM) on tryptophan fluorescence. This higher concentration range was chosen to approximate the known partitioning of volatile anesthetics into hydrophobic regions of membranes (31). At these high concentrations chloroform quenched free t-tryptophan fluorescence to the same degree as it did BSA tryptophan fluorescence. Fig. 4a presents the data in the form of a Stern-Volmer plot, using the equation

$$F/F_0 - 1 = K_{sv} [\text{chloroform}] = \tau_0 / \tau \quad \text{(Eq. 3)}$$

where $K_{sv}$ is the collisional quenching constant, and $\tau_0$ and $\tau$ are the fluorescence lifetimes of tryptophan in the absence and presence of chloroform, respectively (see below). A $K_{sv}$ of 10.4 ± 0.1 M$^{-1}$ was calculated from the slope of the plot.

A linear Stern-Volmer plot can be obtained with either collisional or static quenching (18). Collisional quenching is a diffusion-controlled process that occurs when a quencher molecule is within a certain minimal distance from the fluorophore at the time of excitation. In contrast, static quenching results from the formation of a complex between fluorophore and quencher. To differentiate between these quenching mechanisms, the viscosity of the solvent was increased by adding glycerol. Fig. 4b shows the Stern-Volmer plot of chloroform quenching of tryptophan in 1:1 (v/v) glycerol:methanol. A $K_{sv}$ of 5.6 ± 0.1 M$^{-1}$ was now determined, confirming the collisional nature of the quenching reaction under these conditions.

Fluorescence lifetime analysis was used to differentiate between static and dynamic quenching (32) for the case of protein tryptophan fluorescence quenching by chloroform. A static interaction follows association between fluorophore and quencher, as might be expected with a ligand-protein interaction that involves the fluorophore. Global $\chi^2$ minimization was achieved using biexponential fits with lifetimes of 0.29 ± 0.12 ns and 0.30 ± 0.1 ns for BSA, and 0.41 ± 0.17 ns and 5.4 ± 0.1 ns for HSA. The duration of the long lived component of trypophan fluorescence is comparable to the value of 6.1 ns reported by others for BSA (33, 34), and the value of 5.4 ns for HSA agrees reasonably well with the 5.9–6.0 ns reported previously (33, 35). The preexponential factor ratio was 1:19 for the short and long lifetimes, respectively, for BSA, and 1:7 for HSA. With a biexponential fit, the global $\chi^2$ values were <1.3 for all conditions examined. Assignment of lifetimes in the
presence of chloroform revealed that the longer lifetime component decreased by \(36 \pm 7\%\) at the maximum chloroform concentration (35 mM, Fig. 3d). As seen in Fig. 3, the fluorescence changes measured under steady-state conditions for BSA and HSA (curves a and b, respectively) far exceed the quenching predicted from changes in lifetime data (curves d and c, respectively). These results indicate that a static mechanism is principally responsible for the observed steady-state fluorescence quenching (especially at the lower anesthetic concentrations), implying that chloroform does indeed bind to albumin.

In addition to the proximity of bound chloroform to tryptophan residues, fluorescence quenching might result from structural modifications of BSA upon chloroform binding. Far ultraviolet CD spectroscopy was performed to examine the secondary structure of BSA in the presence of as much as 25 mM chloroform. As shown in Fig. 5, BSA in the absence of chloroform exhibits negative absorption bands with maxima at 222 and 208 nm and a positive band with a maximum at 192 nm, consistent with its highly \(\alpha\)-helical secondary structure (36). Fig. 5 shows that no measurable effect of chloroform on the CD spectrum of BSA was detected.

**DISCUSSION**

The present study demonstrates that intrinsic protein tryptophan fluorescence quenching can be used to monitor the binding of the volatile general anesthetic chloroform. This allows calculation of the energetics of complexation and provides an estimate of the location of the anesthetic in the protein matrix. Furthermore, in conjunction with earlier work (11), the results indicate that chloroform binds to sites on BSA and HSA which are occupied by halothane. Although prior studies using competition assays have suggested that different volatile anesthetics might bind to the same site on a protein (7, 9, 11), this is the first such demonstration using a direct approach to monitor complex formation. This indicates that the clefts, grooves, or cavities on albumin are sufficiently large, or flexible, to accommodate both chloroform (molecular volume \(\approx 102 \, \text{Å}^3\)) using the characteristic atomic volumes of Abraham and McGowan (37)) and halothane (molecular volume \(\approx 129 \, \text{Å}^3\)). This raises the possibility that similar binding domains might also exist on central nervous system proteins that are the actual site(s) of action of the volatile general anesthetics.

The decreased binding of chloroform to the partially denatured BSA in the presence of 50% 2,2,2-trifluoroethanol compared with the binding to the native pH 7.0 form of BSA indicates that tertiary structural features of the protein are critical for the creation of a suitable binding domain. The importance of tertiary structure for the creation of anesthetic binding sites has been implicated in earlier studies using halothane as the ligand (11, 12, 38, 39). The CD studies on BSA in the presence of chloroform indicate that anesthetic binding is not associated with substantial structural changes, in agreement with previous results obtained using halothane (11). This is in accord with other studies on more conventional ligand-receptor interactions (40, 41) which demonstrate minor structural changes in proteins following ligand complexation.

The experiments with myoglobin further demonstrate that chloroform binding to proteins is conformation-dependent. Myoglobin also contains two tryptophans (27). However, the environments of the two indole rings in myoglobin apparently do not favor the binding of chloroform with sufficient proximity or concentration to quench tryptophan fluorescence. This agrees well with prior work (11) that failed to show an association between halothane and myoglobin.

The high degree of quenching of BSA fluorescence by chloroform follows from the partitioning of the anesthetic into hydrophobic domains in the vicinity of the indole rings. Subdomain IIA (containing Trp-214) is known to bind a number of hydrophobic ligands such as warfarin and bilirubin (42). The quenching of free L-tryptophan in methanol in Fig. 4 demonstrates that at sufficiently high concentrations, the degree of fluorescence quenching observed in Fig. 2a for BSA can be attained. This apparent elevated chloroform concentration in the protein matrix is attributed to the favorable energetics of the anesthetic-protein interaction and to steric hindrance to dissociation from the tryptophan residues imposed by the surrounding polypeptide. The fluorescence lifetime studies reveal that chloroform quenches albumin tryptophan fluorescence predominantly via a static mechanism, which indicates that the anesthetic does indeed bind to the protein.

The average binding affinity of the two sites on BSA as reported by intrinsic tryptophan fluorescence quenching is \(2.7 \pm 0.2 \, \text{mM}\). The more solvent-exposed Trp-134 site apparently has a somewhat higher affinity with a \(K_d = 1.5 \pm 0.1 \, \text{mM}\). A prior study on the affinity of chloroform for BSA using a competition approach yielded a \(K_d = 1.3 \pm 0.1 \, \text{mM}\) (9), in good agreement with the present result. The binding constants determined in the present and the earlier study (9) are comparable to the clinical \(EC_{50}\) concentration for chloroform in dogs of 1.2 ± 0.2 mM (1, 43), suggesting that the anesthetic binding domains on BSA may have structural features that mimic the central nervous system sites of action of the general anesthetics, which remain to be described. Albumin may therefore serve as a readily available mammalian protein model for studying the structural features of anesthetic-protein complexes until...
Chloroform Binding to Albumin Using Fluorescence Spectroscopy

Further information regarding the in vivo site(s) of action of the volatile general anesthetics becomes available.

The mechanism of tryptophan fluorescence quenching by chloroform is unclear but may involve electron transfer (29, 30) from the excited indole ring to the anesthetic. Whether the same mechanism is operative in the case of tryptophan residues located in different protein environments remains to be determined. In addition, heavy atom perturbation secondary to the chlorine atoms on the anesthetic may play a role, leading to enhanced rates of intersystem crossing (44). The mechanism is of importance since it may define the proximity of chloroform to the tryptophan residues.

It should be noted that anesthetic-induced structural changes in BSA may cause fluorescence quenching by mechanisms that do not involve close contact between tryptophan and chloroform. A variety of chemical groups present in proteins (including histidine, cysteine, proline, arginine, and the peptide bond) are capable of quenching tryptophan fluorescence (including histidine, cysteine, proline, arginine, and the peptide bond) are capable of quenching tryptophan fluorescence (29, 45), if structural changes alter their proximity to the indole ring. However, chloroform (up to 25 mM) has no effect on the far ultraviolet CD spectrum of BSA, indicating a lack of major secondary structural changes. This result suggests that chloroform interacts with BSA in a manner that does not involve extensive hydrogen bond disruption. It is, however, not possible to exclude the contribution of more subtle structural changes in the protein to the observed fluorescence quenching. Indeed, the finding that volatile anesthetics reverse the chiral nature of bilirubin binding to the subdomain IIA site (46) is presumably the result of a local structural change.

In summary, the volatile general anesthetic chloroform binds to serum albumin in a saturable and reversible manner at, or close to, the sites occupied by the anesthetic halothane. The results suggest that both Trp-134 and Trp-212 are part of the chloroform binding domains, as reported previously for halothane (10, 11), and represent the highest resolution to which such binding sites for chloroform in BSA have been described. This is the first direct study to demonstrate that different volatile general anesthetics may bind to the same site on a protein in solution, providing experimental support for the hypothesis that these structurally diverse drugs may act at similar sites on proteins in the central nervous system.

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