Previous evidence suggests multiple anesthetic binding sites on human serum albumin, but to date, we have only identified Trp-214 in an interdomain cleft as contributing to a binding site. We used a combination of site-directed mutagenesis, photoaffinity labeling, amide hydrogen exchange, and tryptophan fluorescence spectroscopy to evaluate the importance to binding of a large domain III cavity and compare it to binding character of the 214 interdomain cleft. The data show anesthetic binding in this domain III cavity of similar character to the interdomain cleft, but selectivity for different classes of anesthetics exists. Occupancy of these sites stabilizes the native conformation of human serum albumin. The features necessary for binding in the cleft appear to be fairly degenerate, but in addition to hydrophobicity, there is evidence for the importance of polarity. Finally, myristate isosterically competes with anesthetic binding in the domain III cavity and allosterically enhances anesthetic binding in the interdomain cleft.

Serum albumin is the most prevalent soluble protein in humans, and it has a remarkable ability to bind a wide variety of metabolites, toxins, and pharmaceuticals (1). We have shown that binding of the inhaled halocarbon anesthetics to this protein correlates surprisingly well with their pharmacodynamics, suggesting that the binding sites may share features with important targets in the central nervous system (2). Because the identity of those targets is still unrevealed (3), we have used serum albumin as a surrogate to explore the molecular features underlying inhaled anesthetic binding.

We have previously reported that bovine serum albumin binds halothane with dissociation constants of about 1 mM and that human serum albumin binds with about 3–5-fold lower affinity (4, 5). Photolabeling and fluorescence quenching allowed localization of halothane binding to the immediate vicinity of the two tryptophan residues of bovine serum albumin (Trp-214 and Trp-135). HSA\(^*\) has a leucine residue at the analogous 135 position, so the lower affinity of HSA may in part be due to the lack of this tryptophan. However, because both of the experimental approaches used in those studies may overrepresent interactions with tryptophan residues, there may be other halothane binding sites in the two albumin molecules that control the overall effect. Indeed, estimates of binding stoichiometry from photolabeling, \(^{19}\)F NMR, or differential scanning calorimetry studies suggest 3–5 binding sites at saturation, although some of these may be of very low affinity (6–8). One candidate for an anesthetic binding site is a large hydrophobic cavity in domain III that contains tyrosine-411 as a lining residue (9) (Fig. 1). In order to explore this cavity as a new binding site for inhaled anesthetics and compare it to the known interdomain site (Trp-214) also shown in Fig. 1, we used a combination of site-directed mutagenesis, hydrogen exchange, fluorescence spectroscopy, and photolabeling.

**EXPERIMENTAL PROCEDURES**

**Materials**—\(\text{[\textit{14}C]}\)Halothane (51 mCi/mmol) from NEN Life Science Products was stored as a gas in the dark at room temperature. It was condensed and diluted immediately into buffer to make a 7 mM solution immediately before use. Isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether) was obtained from Anaquest (Liberty Corner, NJ), and halothane (1-bromo-1-chloro-2,2,2-trifluoroethane) was obtained from Halocarbon Laboratories (Hackensack, NJ). \(\text{HOCl}\) was obtained from Amer sham Pharmacia Biotech at 100 mM/mmol. The anesthetic (1-chloro-1,2,2-trifluorocyclobutane (F3))/nonanesthetic (1, 2-dichloro-hexafluorocyclobutane (F6)) pair was obtained from PCR Chemical (Gainesville, FL). All other chemicals were reagent grade or better and were obtained from Sigma.

**Synthesis and Purification of Recombinant HSA**—For our studies, we synthesized recombinant wild type HSA (rwtHSA), two single mutants (W214L and W214E), and one double mutant (W214L/Y411W, hereafter called dmHSA). In this latter mutant, placing the tryptophan fluorophore in the 411 cavity permitted monitoring of anesthetic binding by fluorescence quenching (4) and also is expected to enhance halothane photolabeling. Specific mutations were introduced into the HSA-coding region in a plasmid vector containing the entire HSA coding region as described previously (10–12). The experimental methods consist of the following steps.

**Cloning of HSA Coding Region**—With human liver cDNA as template, the entire coding region of the HSA gene, including the native signal sequence, was amplified by polymerase chain reaction using Vent DNA polymerase (New England Biolabs, MA). The resulting DNA fragment was inserted into the plasmid vector pHIL-D2 (In Vitrogen Corp.) using standard cloning techniques. PhII-D2 is a shuttle vector that can be manipulated by cloning in Escherichia coli and that can also be used to introduce genes into the yeast species \(\text{Pichia pastoris}\) (In vitrogen Corp.) by homologous recombination. Specific mutations were introduced into the HSA coding region by using site-directed mutagenesis as described previously (10).

**Expression of Recombinant HSA**—Each pHIL-D2 expression plasmid contained a methanol-inducible promoter upstream of the HSA coding region. For each expression plasmid, a yeast clone that contained the expression cassette stably integrated into the yeast chromosomal DNA was isolated. The native HSA signal sequence, which was left on the HSA coding region, directed high level secretion of mature HSA into the growth medium.

**Verification of DNA Sequence of HSA Clones**—The total genomic DNA from each P. pastoris clone used to produce a particular HSA species was isolated using standard techniques. The genomic DNA isolated from each clone was sequenced using the dyeoxy chain-
Anesthetic Binding Sites in HSA

**RESULTS**

Photolabeling—As expected, total label incorporation into the mutant HSA proteins depended on the presence of a tryptophan residue (Table I). In the rwtHSA, dominant labeling was observed in CNBr-21, the fragment containing Trp-214, and to a lesser degree in CNBr-14, the fragment containing Tyr-411 (Table I and Fig. 2). On transferring the tryptophan from 214 to the 411 position in the dmHSA, there is a clear shift of labeling to this fragment, demonstrating the presence of halothane in this cavity. Labeling of the 14-kDa fragment was clearly inhibited by the presence of saturating myristate more than the 21-kDa fragment was in both the rwtHSA and the dmHSA (Table II). Surprisingly, isoflurane also inhibited labeling of the 411-containing fragment more than that containing 214.

Fluorescence Quenching—With 295 nm excitation, the fluorescence emission of rwtHSA was maximal at 342 nm and was >90% quenched by halothane with an IC$_{50}$ of 3.9 mM in accordance with previous results (4) (Table III and Fig. 3). Isoflurane and F6 also quenched about half of the rwtHSA fluorescence, whereas the tryptophan-containing regions of the dmHSA were less affected by these anesthetics.

**TABLE I**

|        | Intact | CNBr 21 (214) | CNBr 14 (411) |
|--------|--------|---------------|---------------|
| rwtHSA | 100 ± 9 | 100 ± 14      | 100 ± 10      |
| W214E  | 53 ± 10 | 18 ± 2        | 76 ± 11       |
| W214L  | 57 ± 7  | 30 ± 4        | 72 ± 3        |
| DmHSA  | 112 ± 12| 37 ± 7        | 168 ± 10      |

Numbers are % ± S.E. of wild type ratios of reflective density (gel) to absorbance (autoradiogram) of the indicated gel band (n = 3).

**Anesthetic Binding Sites in HSA**

**Photolabeling (14)—**Stock solutions of HSA (4–10 mg/ml) were diluted to ~0.1 mg/ml in 10 mM phosphate buffer, pH 7.4. All photolabeling buffers were first equilibrated with 100% argon. To investigate competitive binding interactions, some samples contained saturating myristate (10-fold molar excess over HSA), and others contained 7 mM isoflurane, an inhaled anesthetic of the ether class. To the protein solution in 2 ml, 0.5-mm-path length quartz cuvettes was added enough [14C]halothane to make a ~0.2 mM solution. This last addition eliminated any gas space from the cuvette, which was then tightly sealed with Teflon stoppers. After ~2 min of mixing equilibration (the myristate-containing samples were incubated for 30 min), cuvettes were exposed to 254 nm light at 5 mm from an Hg(Ar) pencil calibration lamp (Oriel Instruments Inc.) for 30 s, with constant mixing by enclosed microtir bars. Protein was concentrated and washed in Centricon 10-kDa cutoff filters, diluted, lyophilized, and suspended in 70% formic acid with CNBr, 1:1 with protein by mass. After 24 h, this suspension was lyophilized again, suspended in sample buffer, heated, and the CNBr fragments were separated by 16% Tris-Tricine SDS-polyacrylamide gel electrophoresis. Stained, dried gels were placed on Amershaw Hyperfilm to prepare autoradiograms. Incorporate label was normalized to protein mass using a ratio of optical density (autoradiogram) to reflective density (stained gel).

**Fluorescence Spectroscopy—**These inhaled anesthetics contain heavy atoms that can quench tryptophan fluorescence if bound in the immediate vicinity (<5Å) (4). Thus, in order to determine whether the various inhaled anesthetics can gain access to and exhibit selectivity for the tryptophan-containing regions of the rwtHSA or the dmHSA, increasing concentrations of these compounds (from stock buffer solutions) were added to 4-ml fluorescence cuvettes containing ~2 µM protein, with or without a 10-fold molar excess of myristate, and examined in a Shimadzu RF 5301 PC spectrofluorophotometer using 295 nm excitation and emission scanning. Care was taken to eliminate all air from the cuvette with the last addition.

**Hydrogen-Tritium Exchange—**Amide hydrogen-tritium exchange was used to measure binding of anesthetics to HSA (15, 16), because it is a very sensitive measure of shift in the folded/unfolded equilibria, and therefore the effect of an added ligand on the magnitude of the equilibria was constant. Specific binding to the native tertiary structure will shift the equilibrium toward the native conformer, exposing internal amide hydrogens less often and therefore slowing hydrogen exchange with water, whereas nonspecific binding will shift the equilibrium toward less folded conformers, resulting in more rapid exchange of the normally protected amide hydrogens with water hydrogens. For these measurements, protein solutions (10 mg/ml) were incubated with ~5 µCi of $^3$H$_2$O in 1 mM GdnCl, 0.1 mM Na$_2$PO$_4$, pH 8.5, for at least 18 h at room temperature. The GdnCl increased exposure of protected hydrogens to solvent, and the elevated pH increased the rate of chemical hydrogen exchange; both conditions were intended to ensure equilibrium of all exchangeable hydrogens in the protein prior to initiation of exchange-out. Free $^3$H$_2$O was removed and the buffer was exchanged with a PD-10 gel filtration column (Sigma), and exchange-out was thereby initiated. After recovery from the column, the protein solution was immediately transferred to prefilled Hamilton (Reno, NV) gas-tight syringes containing 7.0 mM halothane or isoflurane concentration and solvent conditions and equipped with repeaters (see figure legends). Aliquots were precipitated with 2 ml of ice-cold 10% trichloroacetic acid at timed intervals over at least 6 h. The precipitated protein was rapidly vacuum filtered through Whatman GF/B filters and washed with 8 ml of ice-cold 2% trichloroacetic acid. $^3$H retained by the protein was determined by liquid scintillation counting as above. Exchange-out buffer conditions were adjusted to allow focus on the last 5–10% of hydrogens over a 6-h period, to ensure that global unfolding events were being monitored. Protection factor ratios were determined by dividing the time required for a given hydrogen to exchange-out under the control condition by that required for the same hydrogen of the mutant or anesthetic condition, and ΔΔG was determined using the equation ΔΔG = RT ln(protection factor ratio).

**Amide Hydrogen-Tr%}
emission decrease of only 10.5 ± 1.0% (Figs. 4 and 5). Halothane shifted the emission maxima from 342 to 330 nm, but isoflurane and F3 only shifted it to 337 and 340 nm, respectively, and F6 produced no shift at all. In dmHSA, the fluorescence maxima in the absence of any anesthetic occurred at a significantly shorter wavelength (332 nm) as compared with the rwtHSA, confirming the predicted more hydrophobic nature of this internal cavity as compared with the 214 cleft. Similar to Trp-214, the Trp-411 signal in dmHSA was ~80% reduced by halothane, with an IC₅₀ of 3.4 mM, and a blue shift of only 2 nm (Fig. 3). Maximal concentrations of isoflurane reduced emission intensity by only 30%, with no significant shift in wavelength and an IC₅₀ of about 3.1 mM (Fig. 4). F6 also decreased intensity by ~30% with no wavelength shift, but IC₅₀ could not be reliably calculated (Fig. 5). In contrast, F3 increased fluorescence emission by 20% (Fig. 5). To determine whether the F3 cyclobutane is binding in this 411 cavity in a nonquenching orientation (because it has only a single Cl atom), we examined its ability to reduce quenching by halothane in a competition experiment. Fig. 6 shows that 7 mM F3 was able to inhibit more than 50% of the quenching produced by 2.5 mM halothane in the dmHSA, confirming that F3 binds in this cavity in a nonquenching orientation. The reduced quenching of Trp-411 by isoflurane (also containing a single Cl atom) as compared with Trp-214 may also be due to suboptimal quenching orientation in the cavity, so the relationship between IC₅₀ and KD may be shifted.

As expected, in the W214L and W214E mutants, total fluorescence intensity (280 nm excitation) was much reduced, now predominantly arising from the 18 tyrosine residues. This signal was not significantly altered by up to 10 mM halothane, demonstrating that halothane binding is not so widespread, or the resulting structural changes so large, that tyrosine fluorescence is affected.

Myristate increased the intensity of the fluorescence signal by 33% and shifted the emission maxima from 342 to 333 nm in rwtHSA (Table III and Fig. 3). Halothane was still able to quench Trp-214 fluorescence in the presence of myristate, as shown in Fig. 3, with an essentially unaltered IC₅₀. Isoflurane, on the other hand, quenched the 214 signal more effectively in the presence of myristate, as shown in Fig. 4. Myristate binding enhanced the fluorescence intensity of Trp-411 in the dmHSA by 81% but with no accompanying shift in emission maxima. In contrast to the 214 position, the ability of halothane or isoflurane to quench Trp-411 was essentially eliminated by myristate (Fig. 3).

**DISCUSSION**

These results show that both the 214 cleft and the 411 cavity are occupied by both alkane and ether anesthetics in the low mM concentration range, demonstrating that not only are open pockets or clefts accessible to relatively large molecules, such as isoflurane and cyclobutane (150 Å³), but also apparently inaccessible internal cavities of appropriate volume. The apparent KD values are within an order of magnitude of clinical EC₅₀ values, suggesting that these serum albumin sites may bear some resemblance to those linked to anesthetic action. The binding sites show a modest degree of selectivity for the different compounds. In order of decreasing affinity, the 214 site binds halothane > F3 > isoflurane; and the 411 site binds isoflurane = halothane > F3. Although halothane binds similarly to the two sites, both the fluorescence quenching potency and photolabeling experiments show that isoflurane prefers the 411 cavity to the 214 cleft. This does not appear to depend on the presence of tryptophan, because isoflurane also protected photolabeling of the Tyr-411 fragment in the wild type protein more than Trp-214. Because the 214 pocket is more polar than the 411 cavity (based on tryptophan emission maxima), and because isoflurane is somewhat less hydrophobic than halothane, the basis for this preference is not clear but may relate to a more optimum size of the 411 cavity, providing for better van der Waals interactions. Also, F3 binds only in a nonquenching position in the 411 cavity, whereas it can effectively quench the 214 tryptophan. We interpret this to indicate that the 411 cavity is smaller and F3 is more sterically constrained, whereas a larger 214 pocket allows F3 mobility and hence a greater probability of a quenching orientation, but the necessarily weaker van der Waals interactions should result in lower affinity (higher IC₅₀). We could not confirm this because we could not obtain reliable fluorescence data for F6 binding to the double mutant. Isoflurane may behave more similarly to F3, as reflected by the lower IC₅₀ and degree of Trp-411 quenching, than to Trp-214. F6, the nonanesthetic cyclobutane, minimally occupies either binding site, at least in part due to its exceedingly low solubility in water. In addition to the obvious requirement for hydrophobicity, these results point out the importance of at least two general features of inhaled anesthetic binding sites, volume and polarity.

It is conceivable that the presence of tryptophan in the 411
Anesthetic Binding Sites in HSA

Table II
Label distribution in CNBr fragments in the presence of other ligands

| Molecule, ligand | Slope ± S.E. | IC50, 95% CI | Maximum quenching |
|------------------|-------------|--------------|-------------------|
| HSA, halothane   | -1.4 ± 0.1  | 3.9, 3.4–4.5 | 80%               |
| HSA, isoflurane  | -1.2 ± 0.1  | 12.0, 10.9–13.2 | 87%              |
| HSA, F3          | -1.9 ± 0.1  | 5.4, 4.4–6.6 | 61                |
| HSA, myristate, halothane | -1.0 ± 0.1 | 4.9, 4.2–5.8 | 100%             |
| HSA, myristate, isoflurane | -1.1 ± 0.1 | 6.8, 4.8–9.7 | 87               |
| dmHSA, halothane | -1.1 ± 0.2  | 3.4, 1.9–6.1 | 78                |
| dmHSA, isoflurane | -2.0 ± 0.3 | 2.9, 2.3–3.7 | 28               |

a Bottom values held constant at this value in fit.

Fig. 3. Halothane quenching of tryptophan fluorescence arising from Trp-214 (wtHSA) (top panel) or Trp-411 (dmHSA) (bottom panel) with and without a 10-fold molar excess of myristate. Points are fit with sigmoid curves of variable slope using nonlinear regression (Prism 2), and the parameters are given in Table III.

cavity changes binding energetics relative to wtHSA. Indeed, slight enhancement of stabilization, as reflected by the amide hydrogen exchange results, is consistent with this possibility. However, the similarity of inhibition of photolabeling by halothane and isoflurane in both the dmHSA and wtHSA indicate that the differences are not large and that, even in the presence of the smaller tyrosine, binding in this cavity clearly occurs in the wtHSA.

Based on recent x-ray diffraction studies, myristate has five binding sites on HSA (PDB code 1BJ5), two of which are proximal to the 411 cavity (17). This predicts that myristate should competitively reduce anesthetic binding to this cavity, which both the fluorescence and photolabeling experiments confirm. This is additional strong evidence that places the anesthetics in this cavity and is consistent with the possibility that some components of anesthetic action may be based on competitive interactions with native ligands (18). Although myristate binding produces allosteric effects to the 214 environment, as shown by the fluorescence blue-shift, there is no significant effect on halothane binding in this cleft.

Isoflurane binding, on the other hand, is enhanced in the presence of myristate. Such cooperative binding equilibria may occur in physiologically relevant anesthetic targets. Anesthetic enhancement of agonist binding is thought to underlie the potentiation of inhibitory ligand gated channel activity, and a coupled equilibria dictates that in the presence of agonist, anesthetic binding affinity should also be increased (19, 20). We have confirmed this prediction using autoradiography of photolabeled rat brain slices (21). Also, we and others have previously reported a similar enhancement of anesthetic binding in firefly luciferase on binding ATP/Mg2+ (22) but the structural basis for neither instance is yet clear. The finding of another instance of cooperative binding with a native ligand in this study suggests that this might be a widespread feature of anesthetic interactions with proteins.

The hydrogen exchange results are consistent with the above data in that the folded state is significantly stabilized by low concentrations of these compounds. It is remarkable that the three different residues in the 214 pocket have such small effects on the stabilization of the folded state by anesthetics. This could be due to this binding site contributing only a small fraction of the larger overall stabilization; the results of a similar study (23) is estimated to be less than 4 mM from the fluorescence quenching results. Thus, it is more likely that the interactions necessary for halothane binding in the 214 pocket are fairly degenerate and can be provided by either the leucine or glutamate. It is also possible that the 214 residue does not interact with the bound anesthetic at all, but this is unlikely because heavy atom fluorescence quenching...
is thought to be a close-range event (<5 Å) (4), and removal of the tryptophan 214 residue in the double mutant dramatically reduces photolabeling of the CNBr fragment containing position 214. A final possibility is that the small destabilization of the protein by these mutations may improve access for halothane to other cavities, through increased dynamics and cavity volume distribution, but this seems unlikely because isoflurane stabilization was not similarly enhanced by the mutations.

Given the hydrophobicity of halothane and other inhaled anesthetics, it is somewhat surprising that glutamate provides a more favorable interaction between halothane and HSA as reflected by the hydrogen exchange results. This may indicate a necessity for polarity in the otherwise hydrophobic cavity/cleft, because most potent inhaled anesthetics are known to have an asymmetric distribution of “acidic hydrogens” and a small dipole moment (24). In the wild type protein, several approaches suggest that the CBrClH end of halothane inter-

---

**Anesthetic Binding Sites in HSA**

**Table IV**

| HSA           | Halothane (7 mM) | Isoflurane (7 mM) |
|---------------|------------------|-------------------|
| Wild type     | 1.06 ± 0.2      | 0.90 ± 0.1        |
| W214L         | 1.28 ± 0.1      | 0.74 ± 0.1        |
| W214E         | 1.55 ± 0.2      | 0.92 ± 0.1        |
| W214L/Y411W   | 1.46 ± 0.2      | 0.89 ± 0.2        |

*a* Values are ΔΔG, mean ± S.E. (kcal/mol protein) of two experiments.
acts with the \( \pi \) electrons of the tryptophan residue (25), leaving the trifluoromethyl end to interact with the multiple positively charged residues in this cavity. The glutamate may therefore replace the partial negative charges of the indole ring with a full charge, perhaps providing for improved immobilization of the halothane molecule as compared with the uncharged leucine residue.

The F3 and F6 cyclobutanes were included here in an attempt to further distinguish between the two binding sites and to provide some pharmacodynamic relevance. Both F3 and F6 are hydrophobic and would be expected to produce anesthesia based on the well known correlation of hydrophobicity and potency. Although F3 produces anesthesia in rodents, F6 does not, even at saturating concentrations (102 lower than F3) (26). Because F6 is far less polar than F3, we expected to observe selectivity for these two binding sites, because, clearly, the 411 cavity is less polar than the 214 pocket. In agreement with this prediction, F6 produced some fluorescence quenching of Trp-411, suggesting partial occupancy, but almost no quenching in the more polar 214 site. The anesthetic cyclobutane F3, as well as the clinical anesthetics isoflurane and halothane, bound reasonably well to both the 214 and 411 sites, perhaps with a subtle preference for the cavity. It seems likely that the lack of anesthetic potency in F6 is due to a thermodynamic limitation of binding site occupancy, rather than steric limitations (23).

In summary, at least two distinct binding sites for inhaled anesthetics exist on human serum albumin, one an internal cavity and the other a deep hydrophobic pocket. These sites show low millimolar affinity, are selective for the different inhaled anesthetics, and bind the nonimmobilizer F6 poorly. Occupancy of these sites stabilizes the native conformation of the molecule. The features necessary for binding in the pocket appear to be fairly degenerate, but in addition to hydrophobicity, there is evidence for the importance of polarity. Finally, myristate isostERICally competes with anesthetic binding in the internal cavity and allosterically enhances anesthetic binding in the cleft.

Acknowledgments—We are indebted to Kin Chan for expert technical assistance, to Maryellen Eckenhoff for reviewing the manuscript and assistance with the figures, and to Qing Feng Zhong for preparation of the HSA figure.

REFERENCES

1. Peters, T. (1985) Adv. Protein Chem. 37, 161–245
2. Eckenhoff, R. G. (1998) Mol. Pharmacol. 54, 610–615
3. Eckenhoff, R. G., and Johansson, J. S. (1997) Pharmacol. Rev. 49, 343–367
4. Johansson, J. S., Eckenhoff, R. G., and Dutton, P. L. (1995) Anesthesiology 83, 316–324
5. Eckenhoff, R. G. (1996) J. Biol. Chem. 271, 15521–15526
6. Tanner, J. W., Eckenhoff, R. G., and Liebman, P. A. (1999) Biochim. Biophys. Acta 1430, 46–56
7. Ueda, I., and Yamanaka, M. (1997) Biophys. J. 72, 1812–1817
8. DuBois, B. W., and Evers, A. S. (1992) Biochemistry 31, 7069–7076
9. Sugio, S., Kashima, A., Mochizuki, S., Noda, M., and Kobayashi, K. (1999) Protein Eng. 12, 439–446
10. Petersen, C. E., Ha, C.-E., Mandel, M., and Bhagavan, N. V. (1995) Biochem. Biophys. Res. Commun. 214, 1121–1129
11. Petersen, C. E., Ha, C.-E., Jameson, D. M., and Bhagavan, N. V. (1996) J. Biol. Chem. 271, 19110–19117
12. Petersen, C. E., Ha, C.-E., Harshalli, K., Park, D., and Bhagavan, N. V. (1997) Biochemistry 36, 7012–7017
13. Glatz, J. F. C., and Veerkamp, J. H. (1983) J. Biophys. Methods 8, 57–61
14. Eckenhoff, R. G., and Shuman H. (1993) Anesthesiology 79, 96–106
15. Bai, Y., Mihe, J. S., Mayne, L., and Englander, S. W. (1994) Proteins 20, 4–14
16. Englander, S. W., and Englander, J. J. (1994) Methods Enzymol. 232, 26–42
17. Curry, S., Mandelkow, H., Brick, P., and Franks, N. (1998) Nat. Struct. Biol. 5, 827–835
18. Franks, N. P., and Lieb, W. R. (1984) Nature 310, 599–601
19. Harris, B. D., Moody, E. J., Basile, A. S., and Skolnick, P. (1994) Eur. J. Pharmacol. 267, 269–274
20. Raines, D. E., and Zachariah, V. T. (1999) Anesthesiology 90, 135–146
21. Eckenhoff, R. G., and Eckenhoff, R. G. (1998) J. Pharmacol. Exp. Ther. 285, 371–376
22. Moss, G. W. J., Franks, N. P., and Lieb, W. R. (1991) Proc. Natl. Acad. Sci. USA 88, 134–138
23. Eckenhoff, R. G., Tanner, J. S., and Johansson, J. S. (1999) Mol. Pharmacol. 56, 414–418
24. Scharf, D., and Laasonen, K. (1996) Chem. Phys. Lett. 256, 276–282
25. Dougherty, D. A. (1996) Science 271, 163–168
26. Koblin, D. D., Chortkoff, B. S., Laster, M. J., Eger, E. I., Halsey, M. J., and Ionescu, P. (1994) Anesth. Analg. 79, 1043–1048