technical assistance. T. Collins, L. C. Hayek, N. Knowlton, and H. Lessios gave much useful advice and criticism. The Kuna Nation and the Government of Panama permitted work in San Blas. Supported by the Smithsonian Scholarly Studies and STRI Molecular Evolution Programs.

29 November 1989; accepted 28 February 1990

Anesthesia Cutoff Phenomenon: Interfacial Hydrogen Bonding

JANG-SHING CHIOU, SHAO-MU MA, HIROSHI KAMAYA, ISSAKU UEDA

Anesthesia "cutoff" refers to the phenomenon of loss of anesthetic potency in a homologous series of alkanes and their derivatives when their sizes become too large. In this study, hydrogen bonding of 1-alkanol series (ethanol to eicosanol) to dipalmitoyl-L-α-phosphatidylcholine (DPPC) was studied by Fourier transform infrared spectroscopy (FTIR) in DPPC-D_2O-in-CCl_4 reversed micelles. The alkanols formed hydrogen bonds with the phosphate moiety of DPPC and released the DPPC-bound deuterated water, evidenced by increases in the bound O–H stretching signal of the alkanol-DPPC complex and also in the free O–D stretching band of unbound D_2O. These effects increased according to the elongation of the carbon chain of 1-alkanols from ethanol (C_2) to 1-decanol (C_{10}), but suddenly almost disappeared at 1-tetradecanol (C_{14}). Anesthetic potencies of these alkanols, estimated by the activity of brine shrimps, were linearly related to hydrogen bond–breaking activities below C_{10} and agreed with the FTIR data in the cutoff at C_{10}.

THE ANESTHETIC POTENCY OF 1-ALkanols increases with elongation of their carbon chains, but the potency suddenly disappears when the carbon-chain length exceeds about C_{12} (*1*). No anesthetic potency is observed above C_{14}. This phenomenon is known as "cutoff" and several explanations have been proposed, such as the size of the anesthetic binding site is not large enough to accommodate longer alcohols, or the low water solubility of longer 1-alkanols limits their access to the action sites (*2*). With DPPC vesicle membranes, the main transition temperature between the liquid-crystalline and solid-gel phases decreases by the addition of short-chain 1-alkanols, but increases by long-chain 1-alkanols (*3*). The switchover from depression to elevation of the transition temperature occurs at the same carbon-chain length as the cutoff point. This suggests that the disordering effect of anesthetics on the hydrophobic core of the membrane determines the cutoff phenomenon.

The above result on the phase transition of lipid membranes supports the lipid theory of anesthesia: anesthetics disorder and expand the lipid membrane, and the increased lateral pressure interferes with the electrogenic ionic flow through the ion channels ensuing anesthesia (*4*). Against the lipid theory, which assumes nonspecificity of anesthetic actions, there are proponents for the protein theory who advocate that anesthetics interact with specific receptors of certain proteins (*5*).

We contend that anesthetics interact with macromolecules, irrespective of proteins or lipid membranes, and disrupt water-macromolecule associations. These structures are supported by the hydrogen-bonded matrix of water molecules. Only in water, proteins are formed into meaningful structure or optimal conformations for the assigned biological activities, such as enzyme functions or solute transports. Similarly, lipid membranes are assembled by the interaction with water. When anesthetics disengage the macromolecular structure from the constraint of water matrix, these structures become disordered and expand. The relaxed conformations would be suboptimal for their functions and may be accompanied by global attenuation of biological activities leading to anesthesia. In fact, anesthetized states are characterized by generalized depression of all metabolic activities. Eyring and co-workers proposed that the primary action site of anesthetics is the water-macromolecule interface, releasing electrostricted water molecules from the surface charges with disruption of hydrogen bonds (*6*).

Sandorfy and co-workers (*7*) found in a mixture of secondary aliphatic amines and brominated fluorocarbons that the hydrogen-bonded N–H band decreased when the temperature was lowered and disappeared completely at −190°C, while the free N–H stretching band increased. This was unexpected, because lowering the temperature melted ice. They further showed that volatile anesthetics also disrupt hydrogen bonds and that the anesthetic potency correlates to their hydrogen bond–breaking activity. With 1-alkanols in water-in-oil surfactant-reversed micelles, the association constant between alcohols and the hydrophilic group of the surfactant decreased with elongation

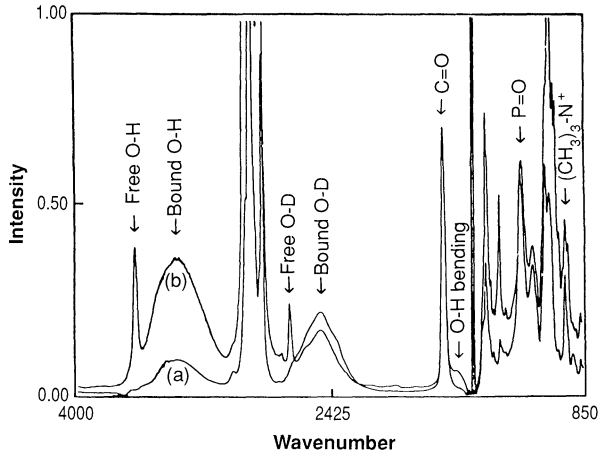
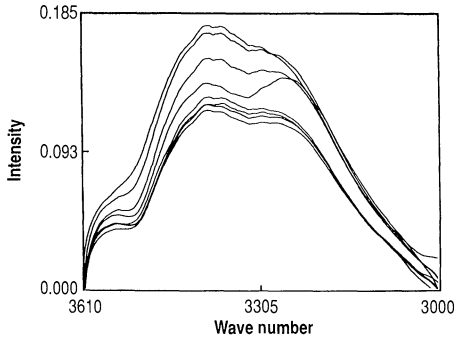


Fig. 1. Difference IR spectra of D_2O-in-CCl_4 reversed micellar systems at 22°C after subtraction of CCl_4 absorption bands: (a) DPPC 0.008*M* in the absence of 1-butanol and (b) DPPC with 0.1*M* of 1-butanol. Synthetic DPPC, D_2O (nominal isotopic purity 100%) and 1-butanol were obtained from Sigma, and spectroscopic-grade CCl_4 from EM Science (Cherry Hill, New Jersey). Carbon tetrachloride was dried by activated aluminum oxide (Fluka) columns. The absence of water was confirmed by infrared spectroscopy. DPPC was kept in a desiccator at reduced pressure until use. D_2O-DPPC-CCl_4 mixtures were sonicated by a Branson ultrasonic disrupter (Danbury, Connecticut) in a cup-horn until clear dispersions were obtained (about 20 min). Each sample was scanned 20 times over the frequency range of 400 to 4000 cm^{-1}. All spectra were acquired at 22.0°C. A Perkin-Elmer model 1750 FTIR spectrophotometer interfaced with a Perkin-Elmer model 7300 computer was used for analysis. The window (FT 04-794) was a fixed-thickness zinc-selenide cell with 1.18-mm pathlength (Spectra-Tech). The cell was calibrated against the benzene band at 845 cm^{-1}. A triglycine sulfate detector was used for all experiments. Assignments of the major peaks are $(CH_3)_3$-N^+ stretching of the choline head at 970 cm^{-1}, P=O stretching at 1238 cm^{-1}, C=O stretching of the ester group at 1734 cm^1, O=D stretching of the free and bound D_2O at 2690 cm^{-1} and 2495 cm^{-1}, respectively, and O–H stretching of the free and bound 1-butanol at 3635 cm^{-1} and 3400 cm^{-1}, respectively. The small absorbance of bound O–H stretching at 3400 cm^{-1} in the control DPPC spectrum (a) may originate from the traces of water contaminating the DPPC and D_2O.

Department of Anesthesia, University of Utah School of Medicine, and Anesthesia Service, Veterans Administration Medical Center, Salt Lake City, UT 84148.

Fig. 2. The effect of 1-alkanols on the O–H stretching band of alkanol-DPPC complex at the frequency range between 3000 and 3610 cm^{-1}. Curves from the top to the bottom are decanol, hexanol, butanol, ethanol, eicosanol, and octadecanol, hexadecanol, and tetradecanol. The order does not follow the carbon-chain length. Alkanols were added to the micellar solution in a 5-ml bottle with a microsyringe under nitrogen gas (closed air-tight by a Teflon cap) and mixed by a vortex mixer. The spectra shown were obtained by subtraction of the spectra of CCl_4 and alkanol in pure CCl_4 solvent. The absorbance maximum was found at about 3400 cm^{-1}.



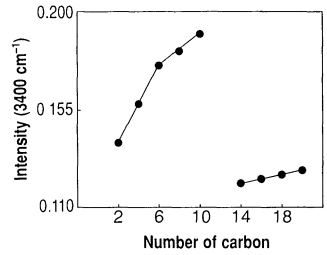


Fig. 3. The effect of the number of carbon atoms in the homologous series of 1-alkanols on the O–H stretching of alkanol-DPPC complex at 3400 cm^{-1}, replotted from Fig. 2. The band intensity increased with elongation of the carbon chain through C_{10}, but suddenly dropped at C_{14}. When the carbon-chain length was further increased beyond C_{14}, the band intensity slightly increased again.

of the carbon-chain length, and then leveled off at C_{10} (7). Our studies (*8*) with Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry, ^1H nuclear magnetic resonance (^1H-NMR), and solution densimetry showed that the interfacial structured water molecules were partially released by the addition of volatile anesthetics. Anesthetic-induced release of electrostricted water molecules from protein surfaces (*8*) was shown in bovine serum albumin and poly-L-lysine by the increase in the partial molal volume. Because electrostricted water structure is almost maximally compressed by the surface charges, disruption of this structure expands the system volume.

In the present study, we used FTIR to examine the effects of carbon-chain length in a homologous series of 1-alkanols from C_2 to C_{20} on the hydrogen bonding to DPPC molecules in a water-in-oil reversed micellar system. By using deuterated water (D_2O) for the aqueous phase, we limited the source of the O–H signal to the OH moiety of 1-alkanols, except for a few water molecules that contaminated the DPPC and D_2O. Carbon tetrachloride (CCl_4) was used for the oil phase to avoid signal interference in the present frequency range. Because the solubility of D_2O in carbon tetrachloride is low and DPPC is hygroscopic, any D_2O molecule added to the system was incorporated into the micelle in close association with the DPPC molecules. This was confirmed by infrared spectra by the absence of free O–D stretching band of unbound D_2O. In the present study, eight D_2O molecules per DPPC molecule were used to prepare the D_2O-in-CCl_4 reversed micelles.

The difference spectra shown in Fig. 1 were obtained after subtraction of the absorption bands of CCl_4 from those of the reversed micelle. Figure 1A shows the control spectrum of 0.008*M* DPPC in D_2O-in-CCl_4 reversed micelles and the assignments of the major peaks of DPPC-D_2O in CCl_4. These assignments agree with the corresponding bands of the phospholipid spectrum previously reported (*9*).

We found that the formation of the alkanol-DPPC complex was saturated at 1-alkanol concentrations between 0.1*M* and 0.2*M*. Hence, 0.1*M* 1-alkanol concentrations were used. Figure 1B shows the spectrum in the presence of 0.1*M* 1-butanol. A sharp new band appeared at 2690 cm^{-1}, which is the free O–D stretching of the released D_2O molecules (*10*). The intensity of this band increased with increasing 1-butanol concentration. Also, the intensity of the broad O–D stretching band of the bound D_2O molecules at 2495 cm^{-1} decreased. The bond between D_2O molecules and the hydrophilic head of DPPC was broken. When DPPC-D_2O was absent, 1-butanol showed only the free O–H stretching band at 3635 cm^{-1}. Addition of DPPC decreased the intensity of this band. The two bands at 3400 cm^{-1} and 1644 cm^{-1} are assigned to the O–H stretching and O–H bending of the hydrogen bonds formed between 1-butanol and the phosphate group of DPPC, respectively. The intensities of these bands increased with the increase in the 1-butanol concentration. Apparently, D_2O-DPPC complex at the interface is replaced by the alkanol-DPPC complex and released D_2O molecules.

Figure 2 shows the difference spectra of the bound O–H stretching region of 0.1*M* 1-alkanols (ethanol, butanol, hexanol, decanol, tetradecanol, hexadecanol, octadecanol, and eicosanol) in the reversed micellar system. The order of the band intensity is clustered in two groups: one group was (from the top to the bottom) C_{10}, C_6, C_4, and C_2, and the other group was C_{20}, C_{18}, C_{16}, and C_{14}. In Fig. 3 the intensity of the bound O–H stretching band of the alkanols at 3400 cm^{-1} is plotted against their carbon-chain length from C_2 to C_{20}. The band intensity suddenly dropped at C_{14}, demonstrating the cutoff phenomenon. The intensity increased again according to the increase in the carbon-chain length above C_{14}. The intensity of C_{20}, however, did not reach the level of C_2. The intensity of the free O–D stretching band also displayed the cutoff phenomenon between the carbon-chain lengths 10 and 14.

The cutoff of the O–H stretching band intensity at C_{14} may not be caused by the increased solubility of long-chain alkanols in CCl_4, because further increase of the carbon-chain length increased the O–H stretching band intensity again. If this were caused by the increased oil solubility of long-chain alkanols, the band intensity should decrease with the increase in the carbon-chain length above C_{14}.

These alkanols may be divided into two groups: anesthetics and nonanesthetics. In each group, elongation of carbon-chain increases the intensity of the O–H stretching band, but there is a large difference in the band intensity between the anesthetic and nonanesthetic groups.

To compare the hydrogen bond–breaking activity with anesthetic action, we determined the anesthetic potency in brine shrimps. Aquatic creatures, such as tadpoles, fishes, newts, and brine shrimps, have been used to determine the anesthetic potencies of alkanols because of the ease of dispensing these drugs. The relation between anesthetic potency as expressed by alkanol concentrations that inhibited brine shrimp motion 50% (ED_{50}) and the intensities of the bound O–H band are shown in Fig. 4. A linear relation was demonstrated between $-\log(ED_{50})$ against log(bound O–H) of 1-alkanol (C_2 to C_{10}). The free O–D stretching band intensity also linearly related to the ED_{50}. The cutoff in anesthetic potency was observed at C_{10}. The ability of alkanols to replace interfacial water correlates with their anesthetic potency and also with the cutoff phenomenon.

Our result shows that the hydrogen bond–breaking activity of anesthetics closely relates to the anesthetic potency. It contradicts the general contention of the lipid theories of anesthesia, which attribute anes-

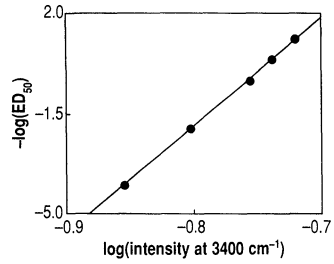


Fig. 4. Linear relation between the logarithm of the O–H stretching intensity in the alkanol-DPPC complex at 3400 cm^{-1} and the negative logarithm of the brine shrimp ED_{50}. Brine shrimps, *Artemia salina*, were captured at the Great Salt Lake in Utah. The brine shrimps' motions were monitored in a 5 by 4 inch shallow glass container by a video camera, interfaced with a digital video adapter XV-D300 (Sony) and an A-D converter, and recorded on a floppy disk of an NEC PC-9801 computer. Their position in the *x-y* plane was digitized every 0.5 s, and 50 data points were analyzed for each scan. The alkanol concentration that reduced shrimp movement to 50% of the control without alkanols was interpolated and designated as ED_{50}. Long-chain alkanols were dissolved in a small amount of ethanol and added to the aqueous phase. The solvent ethanol concentration was well below the anesthetizing concentration.

thetic actions to the conformational change of the lipid tails, hence, the membrane core property. Nevertheless, interfacial hydrogen bond–breaking action of anesthetics is shown in various systems. Release of surface-bound water by anesthetics has been shown in proteins, nonionic and ionic surfactant micelles, phospholipid vesicles, and multilamellar phospholipid suspensions (*8*). Because of the high polarizability, apolar anesthetics, such as cyclopropane, are also shown to break hydrogen bonds (*7*). The increased hydrophobicity of the interface caused by dehydration, together with the conformational relaxation of membranes and proteins, appears to be the cause of anesthesia. The precise mechanism of how these changes induce the state of unconsciousness remains to be elucidated.

REFERENCES AND NOTES

1. K. H. Meyer and H. Hemmi, *Biochem. Z.* **277**, 39 (1935).
2. L. J. Mullins, *Fed. Proc.* **27**, 898 (1968).
3. A. G. Lee, *Biochemistry* **15**, 2448 (1976); M. K. Jain and N. M. Wu, *J. Membr. Biol.* **34**, 157 (1977).
4. P. Seeman, *Pharmacol. Rev.* **24**, 583 (1972); J. R. Trudell, *Anesthesiology* **46**, 5 (1977); T. J. O'Leary, *Biophys. Chem.* **15**, 299 (1982); K. W. Miller, L. L. Firestone, J. K. Alifimoff, P. Streicher, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1084 (1989).
5. F. S. LaBella, *Can. J. Physiol. Pharmacol.* **59**, 432 (1981); N. P. Franks and W. R. Lieb, *Nature* **300**, 487 (1982); A. S. Evers, B. A. Berkowitz, D. A. d'Avignon, *ibid.* **328**, 157 (1987); N. P. Franks and W. R. Lieb, *ibid.* **333**, 662 (1988).
6. H. Eyring, J. W. Woodbury, J. S. D'Arrigo, *Anesthesiology* **38**, 414 (1973); H. Kamaya, I. Ueda, H. Eyring, in *Molecular Mechanisms of Anesthesia: Progress in Anesthesiology*, B. R. Fink, Ed. (Raven Press, New York, 1980), vol. 2, pp. 429–433.
7. T. Di Paolo and C. Sandorfy, *J. Med. Chem.* **17**, 809 (1974); P. Hobza, F. Mulder, C. Sandorfy, *J. Am. Chem. Soc.* **103**, 1360 (1981); *ibid.* **104**, 925 (1982); L. Wilson, R. B. de Alencastro, C. Sandorfy, *Can. J. Chem.* **63**, 40 (1985); P. Menassa and C. Sandorfy, *ibid.*, p. 3367.
8. S. Kaneshina, I. Ueda, H. Kamaya, H. Eyring, *Biochim. Biophys. Acta* **603**, 237 (1980); A. Shibata, H. Kamaya, I. Ueda, *J. Colloid Interface Sci.* **90**, 487 (1982); I. Ueda and T. Mashimo, *Physiol. Chem. Phys.* **14**, 157 (1982); T. Yoshida, H. Okabayashi, K. Takahashi, I. Ueda, *Biochim. Biophys. Acta* **772**, 102 (1984); I. Ueda *et al.*, *Mol. Pharmacol.* **29**, 582 (1986); T. Mashimo, H. Kamaya, I. Ueda, *ibid.*, p. 194; Y. S. Tsai, S. M. Ma, H. Kamaya, I. Ueda, *ibid.* **31**, 623 (1987); T. Yoshida, S. Kaneshina, H. Kamaya, I. Ueda, *J. Colloid Interface Sci.* **116**, 458 (1987); I. Ueda, *Colloids Surfaces* **38**, 37 (1989).
9. D. G. Cameron and H. H. Mantsch, *Biochem. Biophys. Res. Commun.* **83**, 886 (1978); R. L. Amey and D. Chapman, in *Biomembrane Structure and Function*, D. Chapman, Ed. (Macmillan, London, 1983), pp. 199–256; H. L. Casal and H. H. Mantsch, *Biochim. Biophys. Acta* **779**, 381 (1984).
10. J. R. Scherer, M. K. Go, S. Kint, *J. Phys. Chem.* **77**, 2108 (1973); J. Schiffer, M. Intenzo, P. Hayward, C. Calabrease, *J. Chem. Phys.* **64**, 3014 (1975).
11. This study was supported by NIH grant GM25716 and GM27670, and the Veterans Administration Medical Research.

1 December 1989; accepted 5 March 1990

A Mn^{2+}-Dependent Ribozyme

V. DANGE, REUEL B. VAN ATTA, SIDNEY M. HECHT*

An RNA hairpin identical in sequence with the one formed during autocyclization of the 414-nucleotide *Tetrahymena* intervening sequence undergoes strand scission at a specific site in the presence of Mn^{2+}. In addition to representing one of the smallest and simplest ribozymes possible, strand scission occurs readily under physiological conditions, is unaffected by the presence of Mg^{2+}, and displays salt, *p*H, and temperature optima of potential use in exploiting Mn^{2+} as a regulatory switch in intact cells. The chemistry of strand scission of the RNA hairpin is described, as is the Mn^{2+}-dependent solvolysis of a 231-nucleotide RNA transcript containing this structural motif.

A NUMBER OF RNA CATALYSTS HAVE been discovered in recent years; perhaps the best known is the group I ribosomal RNA intron from *Tetrahymena*, which mediates the formation of mature RNA by self-catalyzed processing of the initial RNA transcript (*1*). Cleavage and ligation involve Mg^{2+}-dependent transesterification with nucleophilic attack at the phosphodiester bond by an external (*2*) or internal (*3*) guanine nucleotide 3′-OH group, with concomitant release of an RNA strand possessing a terminal 3′-OH group. Viroid, virusoid, and satellite RNAs from a number of sources (*4, 5*) and human hepatitis delta virus (*6*) also undergo self-catalyzed cleavage in site-specific, Mg^{2+}-dependent processes. Cleavage of the phosphodiester bond occurs by nucleophilic attack of the adjacent 2′-OH group. The products, which include an oligonucleotide 2′,3′-cyclic phosphate and a 3′-fragment having a 5′-OH terminus, are identical with those formed when RNA strand scission is mediated by alkaline hydrolysis, aqueous Pb^{2+} or Zn^{2+}, or some ribonucleases (*7, 8*). Relatively small oligonucleotides have been made that mimic the catalytic behavior of the plant RNAs (*9*); a few of these have been designed to effect sequence-specific cleavage of target RNAs (*9, 10*).

Our interest in defining novel chemical strategies for polynucleotide strand scission prompted us to focus on the role of Mg^{2+} in RNA processing and on the possible involvement of other metals. The foregoing processes all require Mg^{2+}, and proposed reaction intermediates (*11–13*) suggest its direct participation in processes such as (i) activation of phosphorus toward nucleophilic attack, (ii) correct spatial ordering of reaction components, and (iii) general acid-base catalysis. Polynucleotides can bind Mn^{2+} (*14*), and Mn^{2+} may sometimes substitute for Mg^{2+} in RNA processing systems (*8, 9, 13*), consistent with its similar ionic radius and divalent charge. However, unlike Mg^{2+}, which binds to RNA through phosphate oxygens, Mn^{2+} can form complexes involving both the phosphate oxygens and RNA bases (*15*).

Because RNA structure per se can contribute to the feasibility of processing, we sought to identify a structural element that might be stabilized by Mn^{2+} binding or hydrolysis. In this context, the 15-nucleotide (nt) hairpin excised from the 5′ end of the 414-nt *Tetrahymena* intron during autocyclization (*1*) was of special interest. In addition to its formation by Mg^{2+}-catalyzed phosphoryl transfer at the 3′ end (*1*), it was anticipated (*16*) that the hairpin would have

Departments of Chemistry and Biology, University of Virginia, Charlottesville, VA 22901.

*To whom correspondence should be addressed.