Effect of volatile anesthetics on the circular dichroism of bilirubin bound to human serum albumin

A. F. McDonagh[a], Y.-M. Pu[b] and D. A. Lightner[b]

[a] *The Liver Center, Gastrointestinal Research Unit, University of California San Francisco, San Francisco (California 94143-0538, USA), and* [b] *Cell and Molecular Biology Program, Department of Chemistry, University of Nevada, Reno (Nevada 89557-0020, USA)*

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Abstract. The characteristic circular dichroism of bilirubin bound to human serum albumin undergoes a remarkable sign inversion on addition of halothane, chloroform and other volatile anesthetics. This sign inversion, which is completely reversed by removal of the anesthetic, reflects a pronounced conformational change of the bound ligand; probably a complete inversion of chirality. The observation suggests that association of volatile anesthetics with proteins can markedly alter the internal topography of receptor sites and potentially influence the stereoselectivity of ligand binding.

Key words. Bilirubin; albumin; halothane; anesthesia; circular dichroism; exciton coupling.

Bilirubin, the end product of heme metabolism in mammals, is a yellow tetrapyrrole of molecular weight 584 (fig. 1) It is transported in blood as a high-affinity association complex ($K_A \approx 10^8\ M^{-1}$) with albumin [1]. In water at physiologic pH, the complex between bilirubin and human serum albumin (HSA) exhibits a broad UV-visible absorption band near 460 nm and an intense bisignate circular dichroism (CD) spectrum which is characterized by a negative, short-wavelength Cotton effect followed directly by a positive, longer-wavelength Cotton effect [2]. During studies on the mechanism of phototherapy for neonatal jaundice, we noticed that the CD spectra of HSA complexes of bilirubin and its photoisomers are highly sensitive to the presence of chloroform. This led us to investigate the utility of bilirubin as a probe for protein-anesthetic interactions. When we equilibrated a solution of bilirubin (0.22 mM) in phosphate-buffered HSA (0.22 mM, pH 7.4) with halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) in the dark at room temperature and atmospheric pressure the signs of the bisignate CD spectrum inverted (fig. 2). When the halothane was allowed to diffuse out of the solution, the CD spectrum gradually reverted to its original shape, generating a set of curves with a tight isosbestic point near 425 nm. These large changes in CD were accompanied by only minor changes in the absorption spectrum. The same phenomenon was observed when equimolar solutions of bilirubin and HSA were shaken or titrated with halothane, and on removal of dissolved halothane by mild aspiration the CD reverted completely. In contrast, saturation of HSA solutions with halothane in the presence or absence of bilirubin had little effect on the protein CD spectrum (fig. 2). Chloroform, methylene chloride, ether, and ethyl acetate had qualitatively similar effects on the CD spectrum as halothane (table), demonstrating the generality of the effect and showing that the chiral center in halothane [3] is not responsible. Surprisingly, carbon tetrachloride did not invert the CD; nor did the addition of water-miscible organics such as acetone, ethanol, or dimethyl sulphoxide.

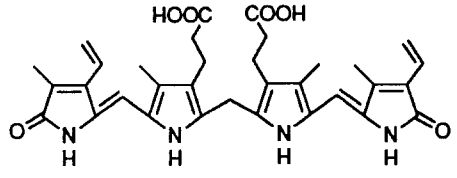

Figure 1. Linear representation of the constitutional structure of bilirubin.

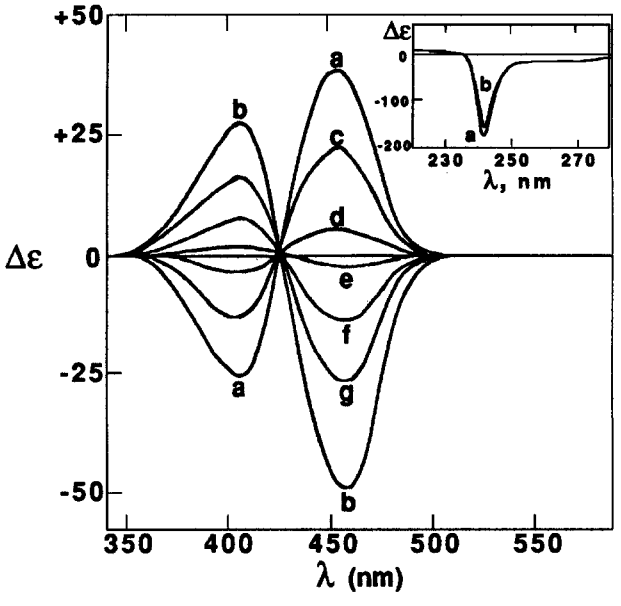

Figure 2. Effect of halothane on the circular dichroism spectra of human serum albumin *(inset)* and on the equimolar complex of bilirubin with human serum albumin. *a* Bilirubin (0.22 mM) and human serum albumin (0.22 mM) in 0.1 M phosphate buffer, pH 7.4. *b* Previous solution after stirring for 8 h in a closed container containing an open vessel of halothane. No further change was seen on continuing exposure to halothane for a further 16 h. *c–g* Spectra obtained on mixing solutions from *a* and *b* at volume ratios of 4:1, 3:2, 1:1, 2:3 and 1:4, respectively. A similar set of curves was obtained when solution *b* was stirred in air and spectra were recorded at intervals as halothane diffused out of the solution. *inset*, CD spectra of a 0.22 mM human serum albumin solution before and after saturation with halothane as in *b*. All spectra were measured at 25 °C in 1-mm pathlength quartz cuvettes.

Effect of volatile anesthetic agents on the circular dichroism and UV-visible spectra of bilirubin bound to human serum albumin

Agent	Concentration (M)	Circular dichroism $\Delta\varepsilon$ (λ_1 nm)	λ_2 at $\Delta\varepsilon = 0$ (nm)	$\Delta\varepsilon(\lambda_3$ nm)	UV-visible ε (λ_{max} nm)
None	0	+38.5 (453)	422	−25.5 (403)	48000 (460)
Halothane	1.75×10^{-2}	−49.5 (455)	427	+30.0 (405)	48500 (455)
Chloroform	6.21×10^{-2}	−95.0 (462)	429	+54.0 (408)	48000 (457)
Dichloromethane	3.10×10^{-2}	−85.0 (460)	427	+50.0 (405)	49000 (455)
Diethyl ether	7.5×10^{-1}	−36.0 (455)	427	+18.5 (405)	49000 (455)
Ethyl acetate	9.3×10^{-1}	−16.0 (455)	427	+ 9.5 (405)	47000 (455)

Bilirubin and albumin concentrations were 2.2 mM in 0.1 M phosphate buffer, pH 7.4. The concentrations of anesthetic agents used were equivalent to their saturation concentrations in pure water[25].

The intense CD of the bilirubin-HSA complex originates from the chirality of the bound pigment[4]. Although bilirubin has no chiral center, it can adopt chiral conformations that can interconvert with their mirror images in solution. Some of these folded chiral conformers are stabilized by intramolecular hydrogen bonding[5], as depicted in figure 3. The conformation of bilirubin when bound to HSA is not known, but folded conformations similar to those observed in crystalline bilirubin[6,7] (fig. 3) are likely. Although bisignate CD curves can be misleading[8], there is strong evidence[4,9,10] that the characteristic sigmoidal shape of the bilirubin spectrum reflects exciton coupling[11] between the two dipyrrinone chromophores of the bound pigment, and that the signed order of the two Cotton effects depends on the relative helical orientation of the electric transition dipole vectors associated with each chromophore[10]. Binding to the protein is enantioselective, and for bilirubin bound to HSA a conformation with *P* (plus), rather than *M* (minus), helicity is favored[4]. Previous studies have shown that halothanes and other volatile anesthetics associate nonspecifically with serum albumins[12–16]. The halothane-induced inversion of the bilirubin-HSA CD spectrum must, therefore, be caused by a change ($P \rightarrow M$) in the relative helicity of the transition dipole vectors – a change that must originate from a pronounced alteration in the stereochemistry of the bound pigment. The nature and cause of this stereochemical change is unclear. One possibility is that halothane displaces bilirubin from its primary binding site to a secondary site at which the pigment binds with inverted chirality. However, this explanation is unlikely for three reasons. First, the halothane effect occurred at bilirubin: HSA mole ratios of not only 1:1, but at ratios of 1:2 and 2:1 also. Second, the CD of bilirubin bound to secondary binding sites on HSA is not inverted with respect to that of bilirubin bound to the primary site. Third, as noted by a reviewer, bilirubin was present at a concentration about four orders of magnitude greater than its dissociation constant, which would require an unlikely binding constant for halothane in the micromolar range for effective competition. A second, more obvious, explanation is that halothane binding causes complete chiral inversion of the bound chromophore, perhaps by changing the conformation of the protein binding site in such a way that a mirror-image conformer of the chromophore is preferentially bound. However, it is also possible that halothane binding induces a conformational change in the chromophore that is sufficient to reverse the helicity of the associated transition dipole moments but does not produce a mirror-image molecule.

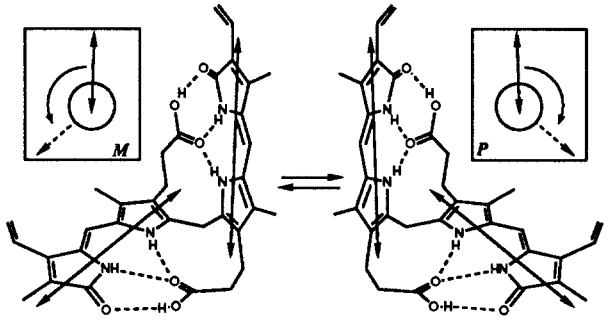

Figure 3. Enantiomeric minimum-energy conformers of bilirubin[26]. Double-headed arrows represent the orientation of the electric dipole transition vectors for the long-wavelength excitation of the dipyrrinone chromophores. The helicity of each pair of vectors is indicated above each structure. Intramolecular hydrogen bonds, represented here by dotted lines, may be perturbed when the pigment, or the corresponding dianion, becomes complexed with serum albumin.

General anesthetics are thought to act by changing the conformation or binding properties of specific neuronal membrane proteins, either indirectly, by dissolving in the surrounding lipid matrix, or by associating with the protein directly[17–20]. Studies have shown that volatile general anesthetics do bind to model proteins such as hemoglobin[21], luciferase[22], and serum albumin[12–14], but, in general, only small structural perturbations have been detected spectroscopically. Although our observations may not be directly relevant to the mechanism of anesthesia, they suggest that volatile anesthetics can potentially have far greater effects on the topography of protein receptor sites than previous spectroscopic studies would indicate[21,23]. Recent work has demonstrated that volatile anesthetics can perturb the binding capacity of HSA for other drugs[14,24]. Our results indicate that anesthetics may also be capable of altering the enantiomeric stereoselectivity of receptor binding sites on HSA and consequently the pharmacokinetics and distri-

bution of co-administered drugs that bind to HSA in blood. They also point to the utility of bilirubin as a sensitive three-dimensional chiroptical probe.

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Editorial note

A. H. Meier and his group are among the few biologists working to understand the implications of the periodicity evident in many physiological systems, such as the circadian changes of blood hormone levels, neuronal activities, and state of energy metabolism. It is known today, due to his and a few others' work, that from teleosts to mammals the daily rhythms of prolactin and corticosteroid secretion seem critically involved in the control of body lipid stores. To manipulate prolactin serum levels, either a prolactin preparation can be injected which in most instances will be heterologous with the consequence of possible differing in the profile of activities from the homologous hormone. Or, endogenous prolactin secretion may be temporarily suppressed by giving a D_2 dopamine receptor agonist, e.g. bromocriptine, accepting the possible complication that after the systemic application such drug's action is possibly not restricted to the inhibitory D_2 receptors of the anterior pituitary prolactin cells, but may affect other dopaminergically controlled systems as well, such as the hypothalamus. Despite these possible complications in the interpretation of experimental findings the results will be of interest to internal medicine.

E. Flückiger

Timed bromocriptine administration reduces body fat stores in obese subjects and hyperglycemia in type II diabetics*

A. H. Meier[a], A. H. Cincotta[b] and W. C. Lovell[c]

[a] *Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge (Louisiana 70803, USA),* [b] *Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge and The Wellman Laboratories of Photomedicine, Massachusetts General Hospital and Harvard Medical School, Dept. of Dermatology, Boston (Massachusetts 02114, USA), and* [c] *Baton Rouge Menopausal Clinic, 545 Colonial Dr., Baton Rouge (Louisiana 70806, USA)*
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Abstract. Obese postmenopausal female volunteers were given timed daily oral dosages of bromocriptine, and tested for reduction of body fat stores. This dopamine agonist has been shown to reset circadian rhythms that are altered in obese animals and to reduce body fat levels in several animal models. The participants were instructed not to alter