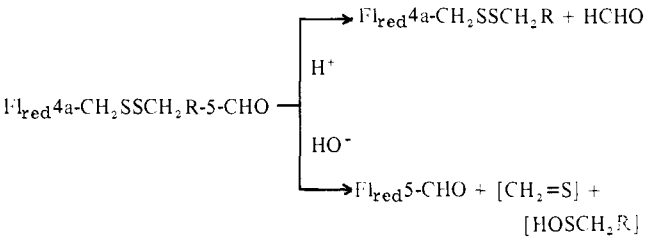

R = Ph, COOMe, COOEt

(44)

$(CH_2)_2S$ with $NaNO_2$[2] and acetic acid to form Fl_{ox} was significantly slower than with either N(5)- or C(4a)-alkylated 1,5- or 4a,5-dihydroflavins.

The disulfides studied herein also undergo acid- and cyanide-catalyzed disulfide exchange to give (Fl_{red}4a-CH_2S–)$_2$.[50,51] The extinction coefficient for this compound at 360 nm is 9550 M^{-1} cm^{-1}, or approximately 1.5 times that for the monomeric compound. The fixed arrangement of the two flavin moieties accounts for this reduced absorption when compared to normal free flavins. This effect was also found by Knappe and Rothfelder[52] for the dimeric forms of dideazaflavins.

Conclusions

Photoexcited 3-methyllumiflavin does not mimic the enzymatic reaction of flavoenzymes with disulfides. Photoalkylation of Fl_{ox} with thioglycolic acid derivatives provides covalent flavin adducts at the 4a-, 5-, 6-, and 8-positions. We have also found that the decomposition of flavin–4a-adducts, to give either Fl_{red} or Fl_{ox}, depends on the electronegativity of the 4a-side chain as has been suggested for the methanol dehydrogenation/formaldehyde reduction by flavins.[53]

Acknowledgment. This work was initiated under the guidance of the late Professor Peter Hemmerich and continued with the hospitality of Professor Wolfgang Pfleiderer. To these individuals we extend our gratitude. This work was also supported by a grant of the Deutsche Forschungsgemeinschaft and of the Studienstiftung des Deutschen Volkes and by the Natural Sciences and Engineering Research Council of Canada.

Registry No. $PhCH_2SSCH_2Ph$, 150-60-7; $HSCH_2CO_2H$, 68-11-1; Fl_{ox}, 18636-32-3; Fl_{red}4a-CH_2SSCH_2Ph, 89322-24-7; Fl_{red}4a-$CH_2SSCH_2CO_2H$, 89322-25-8; Fl_{red}4a-$CH_2SSCH_2C(O)OMe$, 89322-26-9; Fl_{red}4a-$CH_2SSCH_2C(O)OEt$, 89322-27-0; Fl_{red}4a-$CH_2SSCH_2C(O)OMe$-5-CHO, 89322-28-1; Fl_{red}4a-$CH_2SSCH_2CO_2H$-5-CHO, 89322-29-2; Fl_{red}4a-$CH_2SCH_2CO_2H$, 89322-30-5; Fl_{ox}6-$CH_2SSCH_2CO_2H$, 89322-31-6; Fl_{ox}6-$CH_2SSCH_2C(O)OMe$, 89322-32-7; Fl'_{red}4a-$CH_2SSCH_2CO_2H$, 89322-33-8; Fl'_{ox}, 4074-59-3; Fl'_{red}4a-$CH_2SSCH_2C(O)OMe$, 89322-34-9; Fl'_{ox}6-$CH_2SSCH_2CO_2H$, 89322-35-0; Fl'_{ox}6-$CH_2SSCH_2C(O)OMe$, 89322-36-1; (Fl_{red}4a-$CH_2S)_2$, 89322-37-2; Fl_{red}4a,5$(CH_2)_2S$, 89322-38-3; Fl_{red}4a-$CH_2S(O)CH_2C(O)OMe$ (isomer 1), 89322-39-4; Fl_{red}4a-$CH_2S(O)CH_2C(O)OMe$ (isomer 2), 89322-40-7; Fl_{red}4a-CH_2SSBu-*t*, 89322-41-8; Fl_{red}4a-CH_2SCN, 89322-42-9; *N*-bromophthalimide, 2439-85-2; *N*-(benzylthio)phthalimide, 14204-26-3; (benzyldithio)glycolic acid, 83167-33-3; dithiodiglycolic acid, 505-73-7; dithiodiglycolic acid monoethyl ester, 1665-63-0; thiodiglycolic acid, 123-93-3; *tert*-butyl mercaptan, 75-66-1.

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Effect of Hydrostatic Pressure on the Transfer of a Fluorescent Phosphatidylcholine between Apolipoprotein–Phospholipid Recombinants

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Abstract: The effect of high hydrostatic pressure on the mechanism of transfer for lipophiles between membranes was investigated. The transfer of fluorescent phosphatidylcholine (1-myristoyl-2-[9′-(3′-pyrenyl)nonanoyl]phosphatidylcholine) between recombinant complexes of apolipoprotein A-I and 1-palimitoyl-2-oleoylphosphatidylcholine occurred via the aqueous compartment. High hydrostatic pressure retarded the rate of transfer, resulting in a positive activation volume for the reaction. The calculated free energy of activation was approximately 23 kcal/mol. The positive activation volume presumably corresponds to the work necessary to overcome pressure-induced compression of the membrane (protein–lipid complex) before transfer can occur.

The spontaneous passive transfer of phospholipids between lipid compartments (e.g., membranes or lipoproteins) is an important mechanism in metabolism.[1] Kinetic transfer studies of phospholipids,[2,3] in particular, and lipophiles,[4–6] in general, support the mechanism in which dissociation from the lipid surface is the rate-limiting step and the spontaneous transfer of monomeric phospholipid occurs via the aqueous phase. Experimental data suggests that the rate of transfer is a function of the lipid's aqueous solubility.[2] In this communication we report that high hydrostatic pressure (up to 1500 bar) slows the rate of transfer for a pyr-

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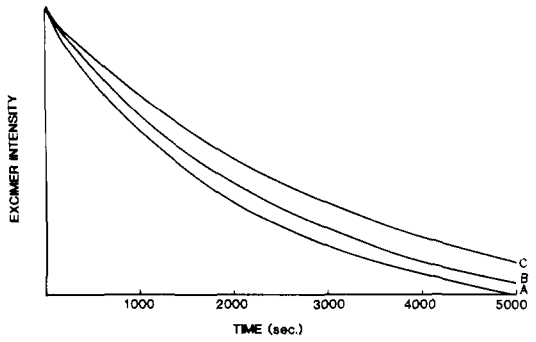

Figure 1. Velocity of MPNPC transfer between POPC/apoA-I complexes at 1 atm (A), 0.5 kbar (B), and 1.0 kbar (C) at 36.7 °C. The excitation wavelength is 345 nm, and excimer emission (arbitrary units) is observed through a Corning 3-70 cutoff filter.

ene-labeled fluorescent phospholipid (1-myristoyl-2-[9′-(3′-pyrenyl)nanonyl]phosphatidylcholine, MPNPC) between complexes of human plasma apolipoprotein A-I (apoA-I) and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) (1:100 M/M).[2] We selected POPC/apoA-I complexes, rather than POPC single bilayers vesicles, because of their greater stability with respect to temperature and pressure and to avoid competitive bilayer equilibration or "flip–flop" reactions (phospholipid $t_{1/2} \sim 3$ h).[7] In general the perturbation of reactions by high pressure results in activation volume changes ($\Delta V^{\ddagger}$) corresponding to a molecular volume change or a reorganization of solvent molecules.[8]

Briefly, the experimental protocol involves mixing donor complexes of POPC/apoA-I containing MPNPC (2 mol %) with a 20-fold excess of acceptor complexes.[2] The decay rate of pyrene excimer fluorescence (475 nm) is a linear function of the rate of change in the microscopic concentration of MPNPC. Transfer kinetics are studied in a pressure vessel similar to that of Paladini and Weber,[9] placed in the thermostated sample compartment of a SLM Instruments 8000 fluorometer. Before mixing the donor and acceptor complexes, the high-pressure vessel and the sample are preequilibrated at the experimental temperature. Mixing of the sample, sealing of the pressure vessel, and pressurization results in a dead time of less than 5 min. The decay data are digitized and the best first-order fit is determined from an iterative procedure.[6] Both donor and acceptor POPC/apoA-I complexes are prepared by the sodium cholate cosolubilization method[10] except that donor complexes contain MPNPC in the lipid suspension. POPC is from Avanti Polar-Lipids (Birmingham, AL), and synthesis of MPNPC has been described previously.[2] ApoA-I is isolated according to published procedures.[11] All transfer reactions are carried out in Tris buffered saline (pH 7.4; 100 mM NaCl, 10 mM Tris, 0.01% EDTA, 0.01% NaN_3), which has a negligible pressure-dependent ionization volume.[12]

The basic equation[13] relating reaction velocity to pressure is

$$k = k_0 \exp[-P\Delta V^{\ddagger}/(RT)]$$

where k and k_0 are the rates at pressure (P) and 1 atm, respectively, and R is the gas constant. At a given temperature T the activation volume $\Delta V^{\ddagger}$ is calculated from the slope of the plot of ln k against pressure.

Figure 1 shows the velocity of MPNPC transfer, expressed as the rate of decay of excimer fluorescence at atmospheric pressure, 0.5 kbar, and 1 kbar. The rate is retarded by pressure. Short-lived transient effects associated with pressurization are not included in the data set for calculating the reaction velocity. Table I summarizes the rate of MPNPC transfer at various temperatures and pressures. In all instances $\Delta V^{\ddagger}$ is positive, which means that the activated state increases in volume, relative to the initial state. The activation volume also increases with temperature, suggesting the correlation between expansivity and volume change.[12] At each pressure the calculated activation energy for MPNPC transfer between POPC/apoA-I complexes is approximately 21 kcal/mol, in good agreement with the data of Massey et al.[2] Calculation of the transition-state thermodynamic values as in ref 5 (Table III) indicates that the primary contribution to the free energy of activation is enthalpic (at 25 °C, $\Delta H^{\ddagger}$ 20.2 kcal/mol, $\Delta S^{\ddagger}$ −9.1 eu, $\Delta G^{\ddagger}$ 22.9 kcal/mol). The empirical correlation between positive $\Delta V^{\ddagger}$ and $\Delta S^{\ddagger}$ for so called "fast" reactions does not apply.[13]

Table I. Transfer of MPNPC between POPC/apoA-I Complexes

T, °C	P, kbar	k, min^{-1} [a]	$\Delta V^{\ddagger}$, mL/mol[b]
30.0	10^{-3}	0.0150	11.8
	0.5	0.0117	
	1.0	0.0108	
	1.5	0.0073	
37.2	10^{-3}	0.0327	12.1
	0.5	0.0267	
	1.0	0.0213	
	1.5	0.0159	
42.0	10^{-3}	0.0568	13.2
	0.5	0.0443	
	1.0	0.0338	
	1.5	0.0261	

[a] Standard deviation of the fit is less than 2%. [b] The standard deviation is less than 5%.

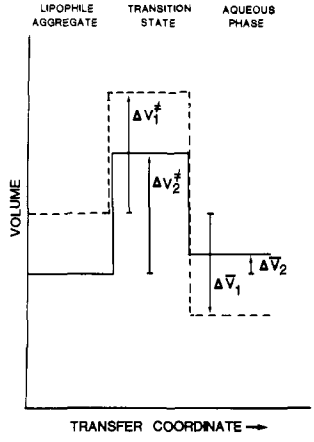

Figure 2. Change in volume of MPNPC associated with transfer from the POPC/apoA-I complex to the aqueous compartment. The subscripts 1 and 2 refer to data at 1 atm and 1 kbar, respectively.

Figure 2 is a schematic representation of the volume change that occurs during transfer of MPNPC from POPC/apoA-I complexes to the aqueous phase. At atmospheric pressure (dashed line) the transfer of a nonpolar molecule from a hydrocarbon solvent to water results in a decrease of the partial molal volume ($\Delta \bar{V}_1 < 0$).[8] However, $\Delta \bar{V}$ for amphiphile transfer[14] is pressure dependent and at approximately 1 kbar (solid line) approaches zero or becomes slightly positive[15] ($\Delta \bar{V}_2 \geq 0$). As our data demonstrate, $\Delta V^{\ddagger}$ is positive and independent of pressure. What is the significance of a positive $\Delta V^{\ddagger}$ for the unimolecular transfer of MPNPC? First, the kinetics of transfer under pressure remain strictly first order, and consequently we consider that the mechanism of transfer through the aqueous phase is maintained. In the temperature–pressure domain we are studying neither apoA-I

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nor POPC undergo a phase transition, since there are no discontinuities in ln k vs. pressure plots.[16] Pressure, however, does compress the host lipid matrix (POPC), and in part the positive $\Delta V^{\ddagger}$ of transfer reflects the work necessary to overcome this effect. Finally, MPNPC is an amphiphilic molecule and changes in volume can arise from electrostriction effects near the charged choline moiety or by hydrophobic hydration of the fatty acid chains. The transfer rates of pyrene conjugated hydrocarbons[4] (uncharged and nonpolar molecules) indicate a $\Delta V^{\ddagger}$ similar to that for MPNPC, correcting for differences in molecular weight (W. W. Mantulin, unpublished data). Therefore, by comparison it appears that the zwitterionic polar head group of MPNPC is not as important a factor as the aliphatic region in establishing the size and magnitude of $\Delta V^{\ddagger}$ for transfer.[17] Since transfer of amphiphiles is governed by "hydrophobic interactions",[14] we postulate a change in the packing of water around MPNPC (hydration density) in the activated state. Future studies to test this hypothesis will focus on the use of neutral salts in a lyotropic series, in conjunction with high-pressure perturbation, to vary lipophile hydration.[18]

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Registry No. POPC, 6753-55-5; MPNPC, 79821-58-2.

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Stereoisomerism and Local Chirality†

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Abstract: The traditional linkage between stereoisomerism and local chirality that is expressed in terms such as "asymmetric carbon atom" or "element of chirality" represents a source of conceptual confusion in modern stereochemistry. Molecular segments must be viewed from two separate and distinct aspects: their character as stereogenic units and their local symmetry. The first is dependent on bonding connectivity (constitution) and is rooted in graph and permutation group theory, whereas the second is independent of constitution and is rooted in the theory of symmetry groups. Although these two aspects are in principle distinct and serve different purposes, they happen to overlap in the case of the regular tetrahedral permutation center. It is for this reason that the concepts of chirality and stereogenicity are most closely associated in organic stereochemistry where this center plays a dominant role. The present analysis clarifies stereochemical concepts, sheds new light on the meaning of stereochemical terminology, and ipso facto disposes of a number of notions introduced into stereochemistry since van 't Hoff's day. To complete our analysis of stereochemical theory, a new treatment of prochirality is proposed. A theoretical framework is constructed that assigns membership in one of three classes of prochirality to any achiral molecular model according to symmetry.

According to van 't Hoff,[1] a carbon atom that is combined with four different univalent groups and whose "affinities" are directed toward the vertices (or, equivalently, faces) of a tetrahedron is "asymmetric".[2] This term refers to the environment of the carbon atom at the center of the tetrahedron, rather than to the atom itself.[3] However, "asymmetric" could obviously just as well refer to the environment of the ligands that are attached to the carbon atom. Why then should this term be reserved for the ligating center? To resolve this question, we must first address the more general problem of symmetry and chirality at the local level.

Local Symmetry

We recently discussed the dissection of geometric objects into isometric segments, with emphasis on objects that represent rigid models of molecules.[5] It was shown that when such an object is partitioned into an ensemble of segments by a cut, the relationship among the segments is dictated by the symmetry of the ensemble, i.e., the object, the cut, and the segments in situ. Intrinsic to this analysis is the restriction that no segment may contain a symmetry element that does not also belong to the molecular model. Two important corollaries from this are that all segments of a chiral model are chiral, and that the segments of an achiral model may be achiral or chiral. Thus, if G and H are the point groups of the model and of any one of its segments, respectively, then H, the local symmetry group, must be a subgroup of G. This condition expresses the fact that every segment must

† Dedicated to the memory of George W. Wheland.

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