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Saturable ethanol binding in rat liver mitochondria

Sreelatha Channareddy, Nhan T. Nguyen, Nathan Janes *

Department of Pathology, Anatomy, and Cell Biology, Medical College of Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107, USA

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Abstract

The binding of ethanol to rat liver mitochondria is shown to be saturable at physiologically relevant ethanol concentrations. This effect is reversible and is not observed in extracted mitochondrial phospholipids. Brief exposure of the mitochondria to heat abolishes saturable ethanol binding. Previously, saturable ethanol binding was reported in rat liver microsomes. Taken together, the studies indicate that saturable ethanol binding motifs may be widespread in cellular membranes. The possibility is raised that incomplete expression of the hydrophobic effect in membrane assembly results in the expression of amphipathic packing defects which display an affinity for and a sensitivity to ethanol. The presence of saturable binding modalities is reconciled with the long-standing consensus on the biodistribution of ethanol – that ethanol's interactions with tissue are negligible – on the grounds that the affinities of ethanol and of water for membranes are similar; consequently, free ethanol concentrations are insensitive to the presence of tissue despite significant ethanol binding. A fraction of the binding sites possess submillimolar affinities for ethanol consistent with published functional studies, both in vitro and in vivo, that reported submillimolar efficacies for ethanol.

Keywords: Alcohol; Anesthetic; Biodistribution; Endogenous ethanol; Micromolar; Specific binding; Submillimolar

1. Introduction

Alcoholism and alcohol abuse are major public health concerns. Excessive alcohol consumption is linked to the injury of many tissues [1]. A wide variety of biochemical processes is altered by ethanol. Neither the pathogenesis of alcoholic tissue injury, nor the origins of ethanol's actions on biochemical processes are well understood [2–4]. In part, this problem stems from a fundamental lack of knowledge about the physicochemical bases by which ethanol interacts with tissue. The consensus from a century of studies on ethanol's biodistribution is that ethanol does not interact significantly with biological tissue [5]. However, recent studies have shown that ethanol, like most organic solvents, binds nonspecifically to lipid membranes, although the binding is weak [6]. Its affinity for other cellular components is poorly understood. The direct observation of specificity in the binding of alcohols and anesthetics long has been thought to be unfeasible due to the large background of lipid solubility for most of these agents. For ethanol, however, the lipid solubility is small and specific binding modalities can dominate the binding trace [7].

* Corresponding author. Fax: +1-215-923-2218; E-mail: nathan.janes@mail.tju.edu

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A dominant force in membrane assembly and structure is the hydrophobic effect and its drive to minimize the exposure of hydrophobic moieties to water and other polar environments. Structural and steric factors, however, compete with the hydrophobic effect and are capable of inducing packing defects or mismatches in polarity that could be ameliorated by the binding of a small amphipathic molecule such as ethanol. In general, cytosolic proteins exhibit tight packing reminiscent of organic crystals [8] and seldom accommodate small nonpolar solutes at physiologically relevant concentrations [9,10]. In those instances where packing defects are expressed, the defects are thought to be of critical functional import in creating a high energy structure prone to facile activation [8,11]. In membranes, where many of the actions of ethanol are believed to be manifest, the structural and functional complexity enhances the probability that hydrophobic packing defects would be formed that could be stabilized by the presence of small amphipathic molecules.

In a previous study, we reported that ethanol binding to rat liver microsomes was saturable [7]. Here, we show that liver mitochondria exhibit saturable ethanol binding. Together, the results suggest that specific binding sites for ethanol are likely to be a common feature of cellular membranes, consistent with the incomplete expression of the hydrophobic effect.

2. Materials and methods

2.1. Membrane samples

Hepatic mitochondria were isolated from chow-fed Sprague Dawley rats (Zivic Miller Laboratories, Allison Park, PA) weighing 350–400 g, as described previously [12] except that the preparation was washed a total of three times in the isolation buffer. The mitochondrial pellet was resuspended at a protein concentration of 8–10 mg/ml and was stored under argon at −20°C. Fractions were monitored by electron microscopy and mitochondrial enrichment was consistent with published studies [13]. Protein was determined by Peterson's modified Lowry method using a bovine serum albumin standard [14]. Thermally treated mitochondria were placed in boiling water for 10 min prior to being subjected to the protocols below. The spin trap α-(4-pyridyl-1-oxide)-*N*-*t*-butylnitrone (POBN) was from Sigma (St. Louis, MO). Cited activities of superoxide dismutase and catalase (Sigma) were as supplied by the manufacturer. Dialysis tubing (12–14 kDa) was from Spectrum (Gardena, CA).

Mitochondrial phospholipids were extracted by the Bligh–Dyer procedure [15] and separated from neutral lipids by silicic acid chromatography (>99%) [16]. Solvents, which contained 0.01% butylated hydroxytoluene to prevent oxidation, were flushed with N_2. Lipids were stored under argon in $CHCl_3$ at −20°C. Multilamellar vesicles were prepared at a concentration of 10 mg/ml by vortexing the dried lipid film with a buffer (10 mM Hepes, 100 mM KCl, pH 7.4).

2.2. Binding assay

Radiolabels were obtained from NEN Research Products, Boston, MA ([^{14}C]ethanol, [^{3}H]water) or Amersham, Arlington Heights, IL ([^{3}H]sucrose). Radiolabels were deemed pure if the partitioning between octanol/water ([^{14}C]ethanol, [^{3}H]water) or butanol/water ([^{3}H]sucrose) reproduced tabulated values [17] and was independent of the bulk solvent ratio at 1:1 and 1:10.

The ethanol binding was determined using a modification of the dual-radiolabel centrifugal technique originally described by Katz and Diamond [18]. Binding to liposomes was performed as described by Janes et al. [19], except that [^{3}H]sucrose (0.1 mM, 1 μCi/ml) was used as a bulk water marker [20] and the binding is expressed as the molal partition coefficient to the hydrated membrane. The binding assay for mitochondria is described below.

Typically, 0.7 ml of the mitochondrial suspension was transferred into a sealed 10 ml Oak Ridge polycarbonate centrifuge tube (Nalgene Co., Rochester, NY), along with 40 μl of [^{3}H]water (9.0 μCi), 12 μl aqueous [^{14}C]ethanol (1.35 μCi), and 4.3 ml of buffer (10 mM Hepes, 100 mM KCl, pH 7.4). The mitochondrial dispersion was equilibrated at 37°C in a water bath for one h, then centrifuged for one h at 37°C at 130 000×*g* in a centrifuge and rotor that were previously equilibrated at 37°C. The rotor temperature was calibrated on a water blank run under

the same conditions. Centrifuged samples were placed immediately in a bath at 37°C. The uncertainty in the temperature throughout the procedure was typically ±1°C. The pellet and four 100-μl aliquots of the supernatant were transferred rapidly into tared glass scintillation vials that were immediately tightly sealed with Polyseal cone caps (Fisher Scientific, Pittsburgh, PA) and again weighed (±0.1 mg). Additional supernatant was occasionally reserved for protein determinations to assess the centrifugal separation (>99%). Typical weights were 100 mg for the supernatants and 45–65 mg for the pellets.

The four supernatant samples were divided into two pairs. To one pair, a nonradiolabeled mitochondrial pellet (~50 mg) was added. Subsequently, 2 ml of tissue solubilizer (TS-2, Research Products International, Mount Prospect, IL) was added to all five vials. The vials were transferred to a 45°C heating bath for at least 20 min for solubilization and then stored in the dark overnight. To the solubilized sample was added 9 ml of Biosafe II scintillation fluid (Research Products International, Mount Prospect, IL) and 1.5 ml of water.

2.3. Liquid scintillation analysis

The radiolabels were counted in a Packard Tri-Carb model 1900CA liquid scintillation analyzer (Packard Instrument Co., Downers Grove, IL) equipped with a barium-133 external γ-ray source, using a dual-window analysis (0–9.1 keV; 9.1–156 keV). The instrument was calibrated weekly. Typical counting times were 5 min.

The presence of membranes imparts a strong color quench to the liquid scintillation analysis. Efforts to decolorize the membrane and maintain the volatile labeled ethanol proved unsatisfactory. Consequently, two sets of quench standards were prepared, counted several times, averaged, fit to a polynomial and stored. Mock samples of known activity were used to ensure discrimination of radionuclei. One set of quench standards was prepared with membranes (generally microsomes, but results with mitochondria were indistinguishable) as a color-quenching agent in the presence of excess water. It was used with the mitochondrial pellets and the supernatants that contained added unlabeled mitochondrial pellet. The other set was prepared with water as a quenching agent for the supernatants that lacked tissue. Equality of the supernatant activities under the two quench curves provided a verification of the counting fidelity.

2.4. Calculation of binding constants

Alcohol binding was calculated according to the approach of Katz and Diamond [18]. The binding was corrected for mitochondrial hydration (nonsolvent water). The reported hydration amounted to 0.4–0.6 μl bound water per mg mitochondrial protein [21] or 0.28–0.42 g bound water per g of dry membrane (with 70% of mitochondrial mass as protein). The mean hydration value of 0.35 for mitochondria was similar to the hydration value of 0.35 that we determined previously for microsomal membranes [7]. Uncertainties in the mitochondrial hydration offset the binding trace by a constant amount, but do not alter the shape of the ethanol binding curve [22]. The ethanol binding constants to mitochondria are molal units, expressed as (mol alcohol in membrane/kg dry membrane)/(mol alcohol in aqueous phase/kg aqueous phase) or equivalently (g alcohol in membrane/kg dry membrane)/(g alcohol in aqueous phase/kg aqueous phase).

3. Results

3.1. Ethanol binding is saturable

The concentration dependence of ethanol binding to the rat liver mitochondria is shown in Fig. 1. Ethanol binding exhibited a steep negative concentration dependence consistent with saturable binding behavior at physiologically relevant ethanol concentrations. The initial and maximum binding constant obtained was 1.2 molal units at 5 μM ethanol (a level comparable to the circulating (endogenous) ethanol levels in abstaining humans (see below)). The decrease in the binding constant was most pronounced to about 25 mM ethanol. From 25 to 300 mM ethanol, the binding constant marginally decreased further. The binding behavior at ethanol concentrations generally regarded as preceding the clinical manifestations of motor impairment (below 2 mM) is shown in the inset. A sharp negative concentration depen-

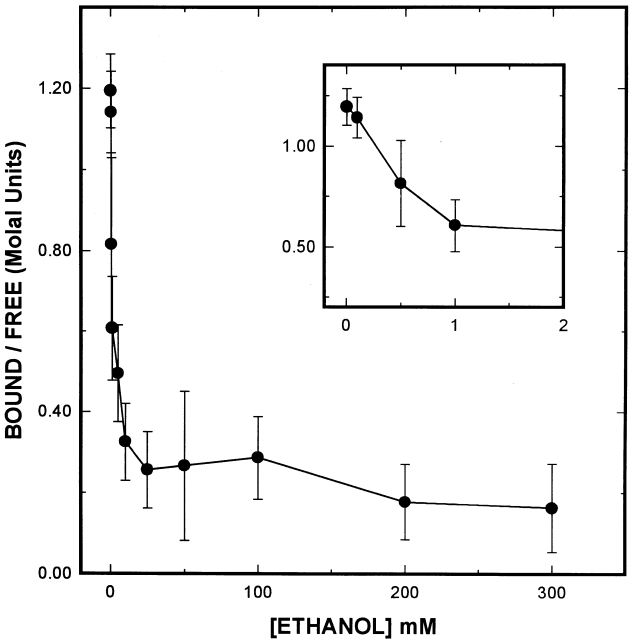

Fig. 1. Saturable ethanol binding in mitochondria. The concentration dependence of the ethanol binding constant (molal units with standard deviation) to the mitochondrial fraction of rat liver at 37°C is shown ($n \geq 5$). The binding behavior at 'subclinical' ethanol concentrations is inset.

dence was evident consistent with the filling of high affinity and low capacity sites.

In Fig. 2, the ethanol binding trace is replotted in terms of the bound ethanol (top trace) over the range of physiological ethanol concentrations in man (0–100 mM). The high-affinity sites for which the binding constant exhibited the steepest concentration dependence possess a modest capacity, whereas the smaller changes in binding constant observed at higher ethanol levels represent a considerably higher capacity. To distinguish between the specific and nonspecific contributions to ethanol binding, the binding at saturation (nonspecific component) is subtracted from the total binding. Given the probability that very high ethanol concentrations were perturbing (see below), the ethanol binding constant at 300 mM was taken as an approximation of the nonspecific binding component. The contributions of the specific and nonspecific components to the total binding are shown in the lower traces of Fig. 2. At ethanol concentrations below approximately 10 mM, the specific binding exceeds the nonspecific binding; whereas above approximately 10 mM the nonspecific component is the larger contribution to the binding. Despite the growth of the uncertainties at the higher ethanol concentrations, the presence of saturable binding sites in the 'subclinical' (0–2 mM ethanol, inset) and the clinical (2–100 mM ethanol) regimes is apparent. Occupancies ranged from approximately 0–0.75 mmol/kg at 'subclinical' concentrations, and ranged from approximately 0.75–10 mmol/kg at clinical concentrations (though the latter value is less certain and sensitive to the correction for nonspecific binding). Thus, specific ethanol binding modalities are expressed in rat liver mitochondria.

3.2. Ethanol binding was reversible

To establish that the saturable binding behavior reflected reversible ethanol binding and not covalent adduction of ethanol (as α-hydroxyethyl, acetaldehyde, or other adducts), ethanol binding was examined using a pulse chase protocol. Mitochondria were incubated for 2 h with trace (5 μM) concentrations of labeled ethanol – a situation where the ethanol binding constant was greatest – followed by incubation

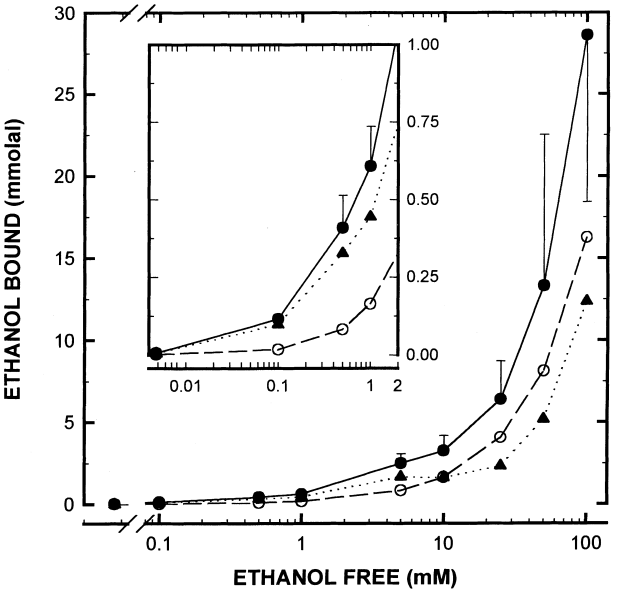

Fig. 2. The concentration dependence of ethanol binding. The binding trace from Fig. 1 is replotted to show the concentration dependence of ethanol binding over the human physiologic range. Shown is the total (●, solid line), specific (▲, dotted line), and nonspecific (○, dashed line) binding. The nonspecific contribution to binding is approximated by the 300 mM point from Fig. 1. The 'subclinical' behavior is inset.

for 2 h with 200 mM ethanol, a situation where the ethanol binding constant was greatly diminished. The rationale being that for reversible processes at equilibrium, the binding constants should not be dependent on the order of addition. In such a situation, the unlabeled ethanol should displace the reversibly-bound labeled ethanol from its binding sites. As shown in Fig. 3, no significant differences in ethanol binding were detected between the pulse-chase methodology and the conventional protocol that employed simultaneous addition of the labeled and unlabeled ethanol.

To reinforce further that α-hydroxyethyl free radicals were not a significant binding pathway, the binding assay was performed with agents that scavenge or block the formation of free radicals from ethanol. The ethanol binding assay was performed in the presence of either (1) the free radical spin trap POBN, or (2) the free radical scavengers superoxide dismutase and catalase. The presence of POBN (32 mM) had no effect on ethanol binding (assessed at 5 μM ethanol where the binding constant was maximal), as was expected for reversible processes. This was a necessary, but not sufficient proof of reversibility, since the spin-trap adduct, if formed, would be radiolabeled and might unexpectedly exhibit binding behavior that mimics binding in its absence. Addition of the free radical scavengers SOD (100 U/ml) and catalase (300 U/ml) to the membrane preparation prior to the addition of ethanol had no effect on ethanol binding. Further corroboration of reversible ethanol binding was evidenced by dialysis of mitochondria incubated with ethanol. Greater than 90% of the ethanol bound at 5 μM ethanol was released after dialysis for 3 h at 37°C with residual slow release noted.

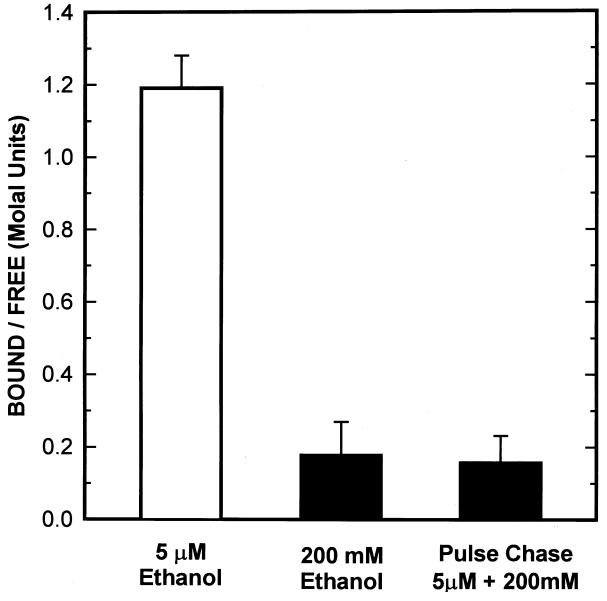

Fig. 3. Saturable ethanol binding is reversed by pulse chase. Ethanol binding at 5 μM (left), 200 mM (center), and 5 μM labeled ethanol followed by equilibration then chased with 200 mM unlabeled ethanol (right), n ≥ 4 with standard deviations.

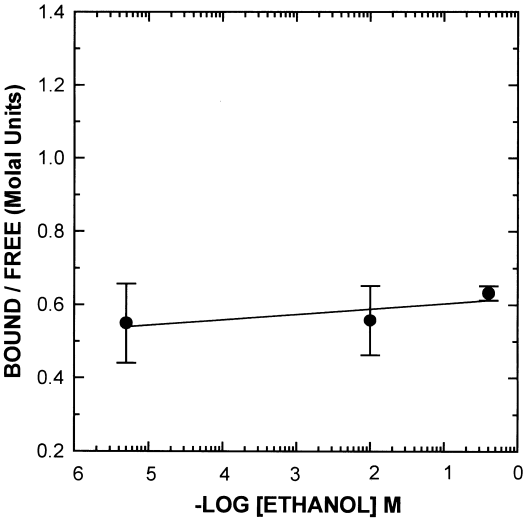

Fig. 4. Ethanol binding in liposomes is not saturable. The concentration dependence of ethanol binding (molal units with standard deviation) to liposomes composed of extracted mitochondrial phospholipids at 37°C is shown (n ≥ 3).

3.3. Ethanol binding in liposomal extracts was not saturable

The binding of ethanol to multilamellar liposomes composed of extracted mitochondrial phospholipids is shown in Fig. 4. No negative concentration dependence that would be indicative of saturable binding was observed in the liposomes. By contrast, a small concentration-dependent increase in ethanol's lipid solubility was observed, consistent with cooperativity in the nonspecific binding. This result supports a nonspecific binding mechanism for the interaction of ethanol with bulk mitochondrial phospholipids.

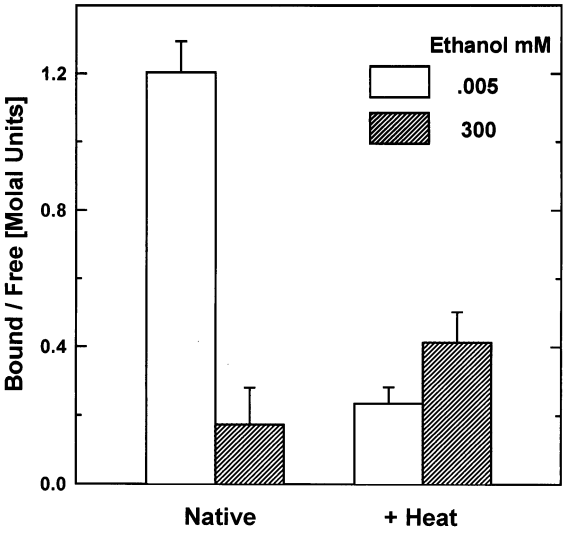

Fig. 5. Thermal treatment abolished saturable binding. The binding of ethanol (molal units with standard deviation) to native mitochondria and to mitochondria subjected to thermal treatment (10 min in boiling water) is shown for ethanol concentrations of 5 μM and 300 mM ($n \geq 4$).

3.4. Saturable ethanol binding was abolished by thermal treatment

To address the role of macromolecular conformation in saturable ethanol binding, mitochondria were subjected briefly to heat (10 min in boiling water), and the binding assay was repeated at 37°C with 5 μM and 300 mM ethanol, as shown in Fig. 5. Thermal treatment appeared to expose additional nonspecific (or innumerable specific) sites. Saturable binding at pharmacologically relevant ethanol concentrations was abolished. A small concentration dependent increase in ethanol binding was observed in the thermally treated mitochondria not unlike that observed in the phospholipid extracts.

4. Discussion

4.1. Reconciliation with established biodistribution data

The biodistribution and binding of ethanol has been studied for over 100 years (reviewed in [5]). Yet, the concept that ethanol binding exhibits considerable specificity in its interaction with tissue is very recent [7]. The historical consensus of the biodistribution studies is that the ethanol content of a given tissue correlates solely with its water content. Biological tissue was shown not to be a significant source of ethanol binding. This concept is best exemplified by a series of studies that employed ethanol as marker for total body water (e.g., [23–25]). These approaches to distribution and binding typically involved the measurement of free ethanol concentrations and the bound ethanol concentration was determined by inference. By contrast, the approach used in the present study assayed the bound ethanol content of tissue directly and thereby accounts for our success in detecting the saturable ethanol binding modalities. Can our data, that shows significant ethanol binding, be reconciled with the scores of studies over nearly a hundred years that show otherwise?

The important concept in reconciling the studies is that both ethanol and water bind weakly to tissue. Being distinct from trapped bulk water, bound water is termed 'nonsolvent water' and from the vantage point of ethanol is inaccessible and not 'aqueous'. Consider the situation where ethanol and water exhibit equal binding constants to tissue. In that case, ethanol would co-distribute between tissue and bulk water equally with the total water so that the addition of dried tissue to an aqueous ethanol solution would not alter the aqueous ethanol concentration (or conversely the ethanolic water concentration). Such a situation is approximately that observed here. From Section 2.4, water binding is about 0.35 g water per g tissue dry wt. The corresponding molal binding constant for water is 0.35 (i.e., (0.35 g water in membrane/g dry membrane)/(g water in aqueous phase/g aqueous phase)). The molal binding constant for ethanol at 25 mM is 0.26 ± 0.09, implying that the ethanol and water distributions in tissue are nearly equal at 25 mM ethanol. Because the binding constant of ethanol is slightly less than that of water, the ethanol concentration in the aqueous phase will increase slightly if added to dried mitochondria. That is, the tissue sequesters more water than ethanol, a situation that is loosely analogous to the formation of ice (i.e., sequestration of water) in alcohol/water mixtures (an approach used to concentrate alcohol in home-made spirits).

Sample calculations may further clarify the point. The total water content of most organs is 0.77 ± 0.03 g/g tissue [5]. As a zero-order approximation of etha-

nol's affinity for the entire organ, we take the extremes in the molal binding constants for mitochondria, from 1.19 ± 0.09 (5 μM) to 0.16 ± 0.11 (300 mM). (This rough approximation seems reasonable because the situation reported previously for hepatic microsomes is qualitatively similar: 5 μM, 0.73 ± 0.14; 25 mM, 0.37 ± 0.20; 400 mM, 0.11 ± 0.09). If one hypothetically reconstitutes 0.23 g dry tissue with 0.77 g of 5 μM of aqueous ethanol, then 0.08 g water is nonsolvent water that is reserved to hydrate the tissue ((0.35 g water/g tissue) times 0.23 g tissue). If ethanol binding had been negligible then the aqueous ethanol concentration would have increased from 5 μM to 5.6 μM. Taking the molal binding constant to be 1.19, however, results in a free ethanol concentration of 4.0 μM for a maximum depletion of the aqueous ethanol content by 20% at the low ethanol extreme. At the high ethanol extreme, the ethanol binding constant is less than the water binding constant of 0.35; consequently, the water sequestered by the tissue as a percentage of the total water is comparatively greater than the ethanol sequestered by the tissue as a percentage of the total ethanol. As a result, the concentration of free ethanol rises. The same calculations for the 300 mM ethanol situation reveals a 6% increase in the free ethanol concentration to 318 mM. This increase belies the fact that at the 300 mM concentration a significant amount of ethanol is bound (ca. 74 mmolal).

Thus, the historical concept that ethanol does not interact significantly with tissue is misleading because it assumes that total tissue water is free water, whereas a significant portion of tissue water is nonsolvent water that itself is bound (i.e., the activity of water is sensitive to the presence of tissue). The likelihood of detecting ethanol binding by traditional approaches should have been greatest in the least hydrated tissues. It is therefore understandable that small, but significant, ethanol binding was detected with the poorly hydrated adipose tissue [26]. We find that the binding constants for ethanol and for water to mitochondria and microsomes are similar. Consequently, the distribution of ethanol mimics that of water so that ethanol serves as a good marker for total body water, and the free ethanol concentrations are rather insensitive to the presence of tissue. In that sense, our results validate the historical consensus that the primary determinant of the ethanol content among the tissues of an organism is the water content of the tissue.

The ability of tissue to deplete the aqueous ethanol concentration has reemerged as an issue in explanations of reduced ethanol visibility in ^{1}H nuclear magnetic resonance spectroscopy. Estimates of brain ethanol visibility ranged from 10% [27] to 100% [28] with most laboratories reporting intermediate values [29–37]. Since the fraction of bound ethanol was unlikely to be detected with the methods used, the extent to which the lack of visibility could be traced to bound ethanol or to alternative factors such as exchange broadening or to instrumental considerations has been debated. From the foregoing work and from the historical consensus, it is evident that the aqueous ethanol concentrations are very weakly dependent on the presence of tissue or the type of tissue; consequently, the source of the signal loss should originate in instrumental or exchange factors. Indeed, the reduced visibility of the ethanol resonances of the high resolution ^{2}H nuclear magnetic resonance spectrum of perdeuterated ethanol equilibrated with liposomal membranes was shown to arise from the latter considerations and not from a depletion of the aqueous ethanol content [6].

4.2. Ethanol binding at submillimolar concentrations

The greatest decline in the ethanol binding constant occurred at what is generally considered as subclinical (<2 mM) concentrations (inset Fig. 1), although the specific site occupancies at these concentrations were low (inset Fig. 2). The biological significance of ethanol concentrations in the micromolar range has been debated. Endogenous sources of ethanol are thought to be formed mainly by alcohol dehydrogenase through the reduction of the endogenous acetaldehyde that, in turn, appears to be primarily generated in glycolysis by pyruvate decarboxylase (reviewed in [38]). Reported endogenous blood alcohol concentrations in human controls ranged from 3 to 10 μM [39,40]. These values are near the lowest ethanol concentration examined in this work (5 μM). In detoxifying alcoholics, however, blood alcohol levels in the range of ca. 10–200 μM correlated inversely with manifestations of the alcohol withdrawal syndrome [40]. In ethanol and water

preferring rats, the levels of endogenous ethanol correlated inversely with ethanol intake [38]; however, the results in abstinent human alcoholics are more contradictory [38,41–44] and possibly confounded by exogenous ethanol sources such as that derived from tobacco use [42] or that from consumption of common soft drinks that were only recently recognized to contain 10–20 mM ethanol [45]. Regarding the latter, a taste preference paradigm indicated that mice were able to distinguish 850 μM ethanol, the lowest concentration tested, from water [46]. In vitro actions of ethanol in the micromolar range also have been reported. The binding of the noncompetitive inhibitor of the *N*-methyl-D-aspartate receptor, MK-801, was stimulated by 0.1–10 μM ethanol [47]. The neuronal nicotinic acetylcholine receptor channel exhibited an EC_{50} of 88.5 μM ethanol [48]. The present evidence of binding sites with a micromolar affinity for ethanol provides a biophysical basis for these functional actions and suggests that other processes may be responsive to the rarely studied 'subclinical' levels of ethanol. Yet, given that exogenous sources of ethanol are common in the human diet (e.g., leavened bread contains 50–200 mM ethanol [49]), the pathophysiological significance of submillimolar levels of circulating ethanol for individuals predisposed to alcoholism requires further exploration.

4.3. Ethanol binding at millimolar concentrations

In the clinical regime (2–100 mM), a smaller decline in the binding constant was evident, but site filling was more substantial as shown in Fig. 1. Specific site occupancies ranged from about 0.75 mmol per kg membrane (dry wt.) at 2 mM ethanol and increased to about 12 mmol per kg at 100 mM ethanol. The estimated protein concentration in mitochondria is roughly 14 millimolal (using 50 kDa proteins that comprised 70% of the mitochondrial mass). Over the clinical range the amount of ethanol molecules specifically bound per protein varied from about 1:20 (at 2 mM free ethanol) to almost 1:1 (100 mM). No evidence for saturable binding or site specificity was found in extracted mitochondrial phospholipids. Previous studies in liposomal membranes supported a nonspecific partitioning process as the binding mechanism for ethanol [6]. Here, a weak positive concentration dependence for ethanol binding to liposomes was observed, a possible indication that higher ethanol levels caused perturbations that cooperatively enhanced binding. A similarly weak positive concentration dependence was reported previously for ethanol binding to microsomal phospholipids [7].

Thermal treatment abolished saturable ethanol binding. If saturable ethanol binding arose from specific binding sites on proteins or at their interfaces, we anticipated that it was likely that those sites would be sensitive to thermal denaturation. Given the amphipathic character of ethanol, its binding sites are likely to involve polar/apolar mismatches or packing defects that are expected to be unstable and sensitive to thermal action. Qualitatively, the binding behavior resembled that observed previously for liver microsomal membranes. Therefore, ethanol binding motifs were not restricted to compositional features unique to microsomal membranes. Rather, it seemed likely that saturable ethanol binding motifs may be widely distributed in biological membranes.

The driving force behind the maintenance of membrane structure is the minimization of polar/apolar mismatches. Any mismatches that remain are likely to be energetically unstable and conformationally labile [50]. Small nonpolar molecules provide a means to ameliorate the mismatches and stabilize the packing defects. These defects may be common in membranes. Specific sites in membranes for nonpolar gases were deduced from the binding behavior of photolytically generated free radical adducts [51]. In abstracted [52] and unpublished work, we have found that a variety of nonpolar solutes compete for the ethanol binding sites in liver mitochondria and microsomes. The binding of those agents themselves was saturable and ethanol was able to compete for their sites.

The results support the concept that defined microstructures within proteins or at their interfaces possess an affinity for ethanol. They raise the possibility that certain motifs presented in biological membranes are inherently ethanol sensitive.

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