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# Ethanol Binding to a Model Carbohydrate, Glycogen

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**ABSTRACT.** The binding affinity of ethanol for carbohydrates is unknown. Glycoconjugates are postulated to be sensitive targets of ethanol action. The glycogen content of muscle, liver, and brain is sensitive to ethanol. To explore whether carbohydrates as a class have a specific affinity to bind ethanol, we measured the binding of ethanol and other small molecules to the carbohydrate glycogen. Ethanol binding was found to be weak. The polar alcohol, glycerol, bound to glycogen with a greater affinity than ethanol did. Other small polar molecules (methanol, sucrose, acetate, glycine, and dimethyl sulfoxide) also bound more strongly than ethanol did. Ethanol and glycerol binding were concentration independent. No evidence of saturable or specific sites for these alcohols was obtained. Water binding was determined and was in agreement with hydrodynamic measures. Water binding exceeded the binding of all solutes studied. The loosely structured water of hydration in glycogen apparently was able to accommodate polar solutes, but tended to exclude ethanol and, to a lesser extent, methanol. We conclude that carbohydrates as a class exhibit no strong affinity or specificity for ethanol.
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**KEY WORDS.** binding; ethanol; glycogen; carbohydrates; anesthetic; hydration; glycerol; alcohol

Carbohydrate moieties of both proteins and lipids are affected by ethanol [1–4] and have been postulated to be sites of ethanol action [2, 3, 5]. Ethanol has been postulated to disrupt the hydrogen-bonding network of membrane glycoconjugates and to alter structure and function [3]. The presentation of glycoprotein carbohydrate moieties at the membrane surface in synaptosomes is thought to be perturbed after chronic ethanol exposure [4]. Glycolipids, especially gangliosides, are implicated as a sensitive target of ethanol [5]. Crystals of the linear polysaccharide amylose are known to accommodate 1-butanol and 1-pentanol [6].

Glycogen is a highly branched polysaccharide that provides a cellular energy store [7]. Ethanol is known to perturb carbohydrate metabolism and, *inter alia*, alter the content of glycogen stores in skeletal muscle [8, 9], liver [10, 11], myocytes [12], and brain [13, 14]. In muscle, the impairment of glycogen deposition caused by acute ethanol exposure is thought to be a direct response to the presence of the ethanol molecule and not a secondary consequence of ethanol's oxidative metabolites or effects on the cellular redox potential [9].

Despite almost a century of effort, the affinity of ethanol for most biological substances is unknown [15]. Due to its weak affinity, binding measurements for ethanol are extremely difficult. Since the aqueous concentration of ethanol is not altered significantly by the presence of biological material, special methods are required that are able to measure the concentration of ethanol in a biological sample and are able to distinguish the ethanol that resides in the substance from the ethanol that resides in the trapped water surrounding the substance.

Early workers correlated the ethanol concentration of tissue with the water content of tissue (reviewed in Ref. 16), even suggesting that whole body water could be determined from ethanol concentrations based on the dilution principle [17]. Later, the binding of ethanol to model phosphatidylcholine membranes was assessed and found to be weak [18] and concentration independent [19]. Modest ethanol binding has been reported in several membrane preparations [20–22], and the values reported were greater than expected from a nonspecific model of ethanol binding based on lipid solubility [23]. Most recently, we observed that ethanol binding in the microsomal membrane fraction of rat liver is saturable [24]. The liver microsomal fraction contains glycogen. Consequently, we wondered whether glycogen possessed an unusual affinity for ethanol, as has been postulated for glycoconjugates, and as might be speculated based on the effects of ethanol on glycogen synthesis.

## MATERIALS AND METHODS

### *Materials*

Glycogen (Type III, rabbit liver), prepared by the aqueous extraction method [25], was obtained from Sigma (St. Louis, MO) and used without further purification (protein 0.1%, glucose < 0.1%, starch < 0.02%, according to sup-

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plier). [$^3$H]Glycerol (DuPont NEN, Boston, MA), [$^3$H]glycine (NEN), [$^{14}$C]methanol [American Radiolabeled Chemicals Inc. (ARC), St. Louis, MO], [$^3$H]sodium acetate (NEN), [$^3$H]dimethyl sulfoxide (ARC), [$^3$H]sucrose (Amersham, Arlington Heights, IL), [$^{14}$C]ethanol (NEN), and [$^3$H]dextran (ARC) were assayed for purity using alcohol/water partitioning [26] at two alcohol/water (octanol/water or butanol/water) ratios (1:1 and 1:10). Pure radiolabels exhibited partitioning that was independent of the alcohol/water ratio. Ethanol titrations were performed with spectroscopic grade ethanol-od (Cambridge Isotope Laboratories, Andover, MA) to ensure the absence of impurities common to commercial ethanol [27]. Glycerol was obtained from Aldrich (Milwaukee, WI).

### *Binding Assay*

Binding was determined using a modification of the dual-radiolabel centrifugal technique described by Katz and Diamond [28] and Janes *et al.* [29]. This approach is suitable for a wide range of solute concentrations. The technique is a ratio method; consequently, it is insensitive to uncertainties in the specific activity of the sample, to evaporation, or to the adsorption of radiolabels to the walls of the sample tubes.

Briefly, radiolabels were added to an aqueous suspension of glycogen (30 mg/mL in 10 mM HEPES, 100 mM KCl). The suspension was allowed to equilibrate at 37° for 1 hr, and the glycogen was pelleted by centrifugation at 37° for 90 min at 130,000 *g*. Pellet and supernatant samples were transferred to tared scintillation vials and weighed. After addition of 1.5 mL of water, the vials were transferred to a 45° heating bath for at least 20 min. Subsequently, 2 mL of tissue solubilizer (TS-2, Research Products International, Mount Prospect, IL) and 9 mL of Biosafe II scintillation fluid (Research Products International) were added. After preparation, the sample vials were stored for 2 days in the dark to achieve a stable quench.

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 $\gamma$-ray source, using a dual-window analysis (0 to 9.1 keV; 9.1 to 156 keV). Quench standards were prepared using water (250-$\mu$L increments centered at 1.5 mL, 9 mL Biosafe, 2 mL TS-2) as a quenching agent. The instrument was calibrated weekly. Typical counting times were 5 min.

Centrifugal separation was monitored by the mass of the lyophilized pellets, and the supernatants were prepared in duplicate without radiolabels and with a correction for buffer salt content. Separations of 97% were achieved.

Accurate determination of binding constants required that the glycogen-bound water in the pellet be distinguished from the bulk pellet water. We modified the approach of Katz and Diamond [30]. $^3H_2O$ was used as a marker for total pellet water. [$^3$H]Dextran (70 kDa) was used as a marker for bulk solvent water. The difference was the water of hydration or bound nonsolvent water. Binding is expressed in molal units [(mol solute in glycogen/kg hydrated glycogen)/(mol solute in water/kg water).

## RESULTS

The concentration dependence of alcohol binding to glycogen is shown in Fig. 1. Over the concentration range from 5 $\mu$M to 400 mM there was no significant concentration dependence for the binding of either glycerol or ethanol. Ethanol binding was weaker than glycerol binding at all concentrations. Preliminary experiments revealed similarly weak ethanol binding to a crude glycogen fraction isolated from rat liver.

The ability of a number of polar agents to bind to glycogen is shown in Fig. 2. Water binding exceeded the binding of all the agents shown. The other polar agents exhibited an affinity for glycogen that did not vary widely and was less than that of water. The less polar normal alcohols had the lowest affinity for glycogen, with the affinity for methanol being slightly greater than that of ethanol.

## DISCUSSION

Glycogen is a highly branched and structurally complex carbohydrate [7]. It is hydrated extensively [31]. In the present study hydration was determined as the water that is inaccessible to dextran (70 kDa) based on steric exclusion. The hydration obtained was 1.3 g/g glycogen. This value is in agreement with the hydrodynamic value of 1.1 g water/g glycogen [31]. To reflect the extensive hydration, binding

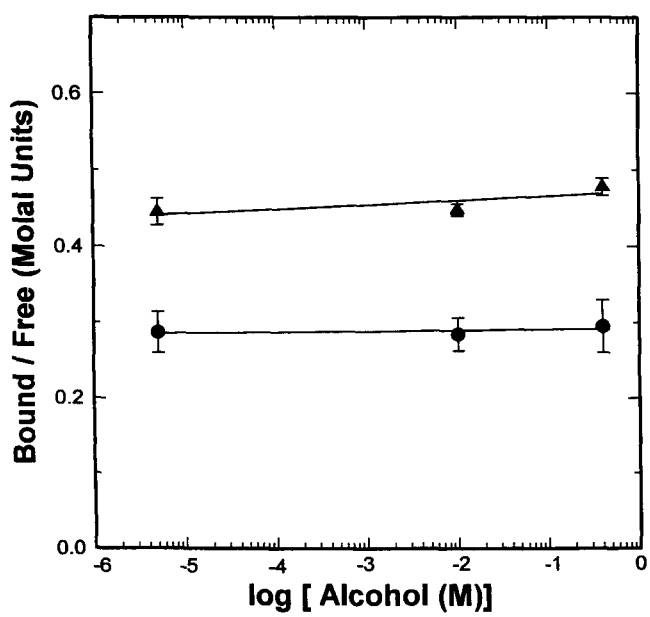


**FIG. 1. Concentration dependence of alcohol binding. The binding of ethanol (●) and glycerol (▲) to rabbit liver glycogen is shown as a function of the respective alcohol concentration and fit by linear regression. Values are means ± SD (N ≥ 4).**

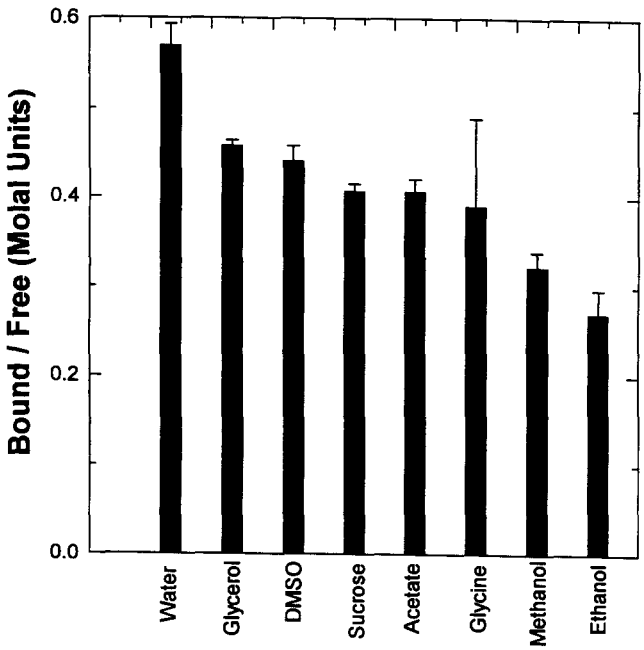


**FIG. 2. Solute binding to glycogen. Molal binding constants for water, glycerol (5 μM), DMSO (10 mM), sucrose (0.05 μM), acetate (25 mM), glycine (0.02 μM), methanol (0.55 mM), and ethanol (4.8 μM) to glycogen are shown. Values are means ± SD ($N \geq 3$).**

constants are presented in terms of the hydrated glycogen particle. Dry weight values are obtained by multiplying these values by 2.3. The water of hydration determined by steric exclusion is anticipated to be a combination of highly structured water that is integral to the glycogen structure and more loosely structured water that occupies the voids in the glycogen macrostructure.

Our results are consistent with the idea that the loosely structured bound water, perhaps together with the glycogen hydroxyls, is able to accommodate polar solutes through a nonspecific process that mimics bulk solubility into this hydration pool. Less polar solutes, such as methanol and ethanol, are less easily accommodated within this loosely structured, but very polar, hydration pool. The low amount of ethanol that was found within the glycogen particle may simply reflect the different steric constraints for ethanol and dextran that determine their accessibility to the most loosely structured intraparticle water.

The affinity of glycogen for ethanol was very weak and less than that of a variety of polar solutes and common metabolites. Such a weak interaction is not consistent with the direct action of ethanol on glycogen. Nor is it consistent with the concept that carbohydrates as a class exhibit unusual affinity or specificity for ethanol.

The situation in glycoconjugates may be more complex. The affinity of glycogen for water is quite strong. The hydrated particle contains more water by weight than carbohydrate. This offers the possibility that in glycoconjugates the carbohydrate moiety could induce indirectly an affinity for ethanol, if strong hydration forces act to present the carbohydrate residue to water at the expense of exposing less polar regions of the membrane or protein to water.

This study was designed to test whether ethanol has an inherent affinity for the extensive hydrogen bonding network that is a ubiquitous feature of complex carbohydrates. It did not. Yet, cellular carbohydrates exhibit great diversity in structure and composition. The highly branched glucose polymer studied here in no way replicates this vast diversity. Our interpretation is not that no carbohydrate has an affinity for ethanol, but rather that the hydrogen bonding network characteristic of carbohydrates as a class does not predispose them to ethanol.

Our results led us to the following conclusions. First, the binding of ethanol and glycerol to glycogen is concentration independent. This is consistent with a bulk solvation process into a pool of loosely structured water or to numerous specific sites on glycogen. Second, the affinity of ethanol for glycogen is weak and less than more polar alcohols like glycerol and sucrose. Third, carbohydrates as a class do not have an inherent affinity for ethanol.

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