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Horse Liver Alcohol Dehydrogenase as a Probe for Nanostructuring Effects of Alcohols in Water/Nonionic Surfactant Systems

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The kinetics of alcohol oxidation catalyzed by the enzyme horse liver alcohol dehydrogenase (HLADH) is studied in water/alcohol/$C_{12}E_{23}$ systems with a series of *n*-alcohols ranging from ethanol to 1-decanol or with α,ω-alkandiols, namely, 1,5-pentanediol and 1,7-heptanediol. Essentially, the water-rich part of the ternary systems is examined, either without $C_{12}E_{23}$ or at several constant $C_{12}E_{23}$ concentrations above the cmc (1, 8, and 22 mass %). The substrate inhibition of the enzyme allows one to infer alcohol partition coefficients between the outer aqueous pseudophase and the surfactant aggregation pseudophase. In the case of short-chain *n*-alcohol (ethanol, 1-propanol) and alkandiol (1,5-pentanediol) systems, the alcohol seems to remain in the aqueous pseudophase, whereas in the case of middle- and long-chain *n*-alcohol (1-butanol to 1-decanol) and alkandiol (1,7-heptandiol) systems, the alcohol participates in the structuration of the micelle.

1. Introduction

Biocatalysis in liquid two-phase systems has attracted increasing interest in the last years.[1] Hydrophobic substrates may be dissolved in the organic phase, whereas the enzyme is in the aqueous solution. A close contact between the two phases can be achieved with the help of emulsifiers. The resulting system can be either an emulsion or a microemulsion. Both types of systems have advantages and disadvantages depending on the special type of reaction to be catalyzed by a special enzyme.

In our laboratory, we try to understand the interplay between enzymatic activity and the aggregation of substrate molecules in direct micellar systems, especially when the substrate participates in the structuration of the liquids. For example, this is the case when substrate molecules play either the role of the organic component or that of a cosurfactant.

In a recent study,[2] it was shown that the enzymatic oxidation of 1-pentanol to pentanal strongly depended on the nanostructuring of the buffer medium. In the case in which direct micelles were formed by the nonionic surfactant $C_{12}E_{23}$ (poly(oxyethylene-23)lauryl ether, commercial name Brij 35), the alcohol was partially dissolved in these micelles and thus the substrate inhibition of the enzyme horse liver alcohol dehydrogenase (HLADH) was significantly attenuated. By contrast, in corresponding ethanol solutions, the enzymatic activity was not significantly affected by the presence of surfactant molecules. To explain the relation between structure and reactivity, small-angle neutron scattering (SANS) experiments have also been carried out. For surfactant concentrations up to 8 mass %, the SANS spectra could recently be modeled quantitatively with the help of Percus−Yevick integral equation calculations and a soft-sphere shell/core model for the micelles.[3] This was possible for binary $D_2O/C_{12}E_{23}$ and ternary $D_2O/C_{12}E_{23}$/alcohol systems, the alcohol being 1-butanol, 1-pentanol, 1-hexanol, and 1-heptanol.

In the present study, we extended our corresponding enzymatic studies to a whole series of *n*-alcohols ranging from ethanol to 1-decanol and to the enzymatic oxidation of two α,ω-alkandiols, namely, 1,5-pentanediol and 1,7-heptanediol. Diols are interesting because they are less toxic than comparable alcohols and they may act as cosurfactants[4,5] or cosolvents.[6−8] The enzyme activities were determined for various $C_{12}E_{23}$ concentrations and alcohol concentrations ranging from nearly infinite dilution up to the limit given by the solubility of the different components in the medium.

It should be mentioned that similar experiments have already been done in reversed micelle systems in which the enzyme was entrapped in the aqueous core and the enzymatic activity could be explained by assuming a partition of the substrates between the continuous organic pseudophase and the aqueous cores of the micelles.[9,10,12−20] Studies of enzymatic activity in aqueous surfactant systems are much harder to find.[2,21−23] However, enzymatic reactions were never used to distinguish between a possible cosolvent and a cosurfactant behavior of alcohols.

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TABLE 1: Used Alkanols and Alkanediols

name	purity [%]	purchased from
ethanol	99.8	Roth
1-propanol	99	Merck
1-butanol	99.7	Merck
1-pentanol	99	Fluka
1-hexanol	98	Aldrich
1-heptanol	98	Aldrich
1-octanol	99	Aldrich
1-nonanol	98	Aldrich
1-dekanol	99	Aldrich
1,5-pentanediol	>98	Merck
1,7-heptanediol	95	Aldrich

The main objectives of the present paper are the following ones: First, we wanted to determine the influence of the constitution and the structuration of the monophasic media water/$C_{12}E_{23}$/alcohol on the activity of HLADH to check the specificity, the potential, and the limits of this enzyme and to compare the results to information from literature on micellar formation of such ternary systems. Second, if there is a relation between structuration and enzyme activity, we wanted to know whether one can use the enzyme as a convenient probe to infer structural changes in the ternary systems. Third, the results of this study should contribute to development of enzymatic bioconversions of industrial interest in aqueous systems without additional organic solvent.[19,20]

2. Materials and Methods

2.1. Materials. The used water was produced with a Millipore Milli-Q system, the electrical conductivity being less than 10^{-6} S m^{-1}. The buffer contained semicarbazide chlorhydrate (purity > 99.5%, Sigma, concentration $c = 0.075$ M), glycine (Sigma, $c = 0.1$ M), or NaOH (purity ≥ 99%, Merck), pH = 8.7. The different alcohols used are given in Table 1. We chose poly(oxyethylene-23)lauryl ether (Fluka, Brij 35 or $C_{12}E_{23}$, CH_3-$(CH_2)_{11}(OCH_2CH_2)_{23}OH$, average molar mass = 1199.8 g/mol; note that there is a certain distribution within the number of oxyethylen groups) as surfactant. The enzyme was horse liver alcohol dehydrogenase (Fluka, HLADH, batches 394514/1 42199 and 71014/1 42500, denoted further on as batches A and B, respectively). Nicotinamide adenine dinucleotide (NAD^+) in its oxidized form (Merck, batch 24343542 750, purity ≥ 95%) acted as cofactor.

2.2. Preparation of the Reaction Mixtures. The reaction mixtures are prepared by adding 25 μl of buffer solution containing 1 g/L of HLADH and then 25 μl of buffer solution containing the cofactor NAD^+ (20 g/L) to 3 mL of the basic host medium. The addition of the cofactor starts the following reaction:

$$R{-}CH_2{-}OH + NAD^+ \xrightarrow{\text{HLADH}} R{-}CHO + NADH + H^+$$

with R being the corresponding hydrocarbon chain ($R = -(CH_2)_{n-2}-CH_3$) of the *n*-alcohols and aldehydes. In the case of the diols, $R = -(CH_2)_{n-1}-OH$. Of course, both OH-groups can be oxidized.

2.3. Determination of Enzymatic Activity. The kinetics of alcohol oxidation was followed spectroscopically in a study, which was carried out with the batches 394514/1 42199 and 71014/1 42500 of HLADH and 24343542 750 of NAD^+ at 25 °C using a Varian Cary 3E spectrometer. The progress of the reaction was estimated from the absorption at 340 nm of produced $NADH + H^+$, detected during the first 3 min after the addition of NAD^+. The initial velocity, *V*, of the enzymatic reaction was inferred from the slope of the absorption intensity versus time, which was linear at least during the first minute.[24] We restricted our experiments to initial velocities to neglect the influence of the produced aldehydes on the nanostructuring, although it seems that aldehydes have a structuring behavior similar to alcohols.[25]

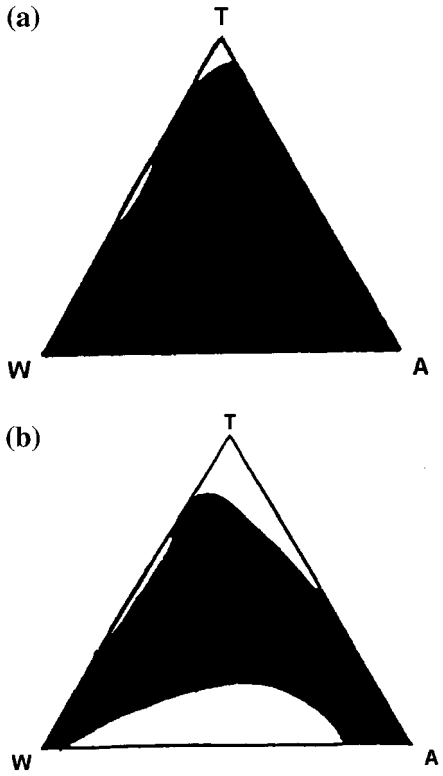

Figure 1. Phase diagrams of ternary $C_{12}E_{23}$/alcohol/water systems (A stands for alcohol, T for surfactant, and W for water) taken from ref 26. Panel a shows the phase diagram for the ethanol and panel b for the 1-butanol system.

Because the continuous phase is water, the enzymatic activity can directly be compared to the corresponding activity in molecular aqueous solutions without micellar structures. Therefore, and to reduce the uncertainty of the measured result due to variations of the purity of the enzyme, the activity, *A*, was defined with respect to the initial velocity, V_0, of the same enzyme reaction in an aqueous ethanol solution (concentration of ethanol is 10^{-2} mol/L ≈ 10^{-5} mol/g of buffer), $A = V/V_0$. V_0 and *V* were determined under exactly the same experimental conditions. For each of the different reaction compositions, at least three identical samples were prepared in each study and the reaction velocities in them were measured independently at 25.0 ± 0.5 °C to verify the reproducibility of the results.

Although in this way the different activities of enzyme coming from different batches were standardized, the absolute values of corresponding activities varied considerably, but the shape and the position of the maximum did not, as can be shown in Figures 3, 5, and 7 for the ethanol and 1-pentanol systems.

3. Results

3.1. Phase Diagrams. Phase diagrams of four ternary systems, water/alcohol/$C_{12}E_{23}$, are given in Figures 1 and 2.[26] Black areas (L) represent realms of existence of monophasic, isotropic, and thermodynamically stable liquids of low viscosity. White areas correspond to other phase types but are not marked here in detail, because we carried out our experiments in the monophasic areas.

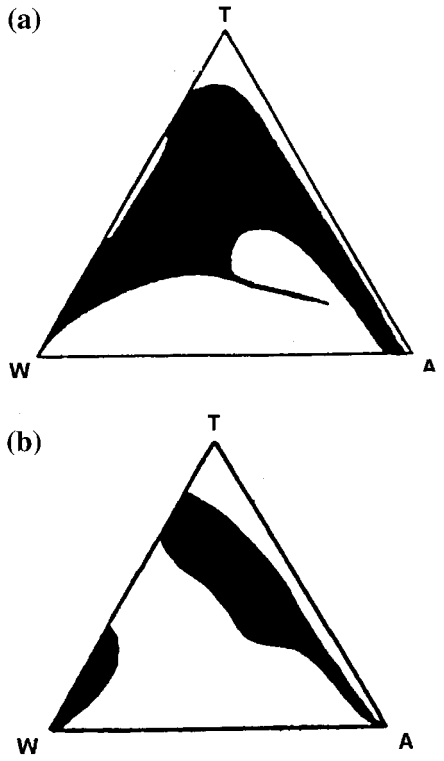

Figure 2. Phase diagrams of ternary $C_{12}E_{23}$/alcohol/water systems (continued). Panel a shows the phase diagram for the 1-hexanol and panel b for the 1-nonanol system.

Because the phase behavior is almost the same or very similar for ethanol and 1-propanol, for 1-pentanol, 1-hexanol, and 1-heptanol, and for 1-octanol, 1-nonanol, and 1-decanol,[26] we show only the phase diagrams for four alcohols, namely, ethanol, 1-butanol, 1-hexanol, and 1-nonanol.

3.2. Preliminary Tests. In a first series of experiments, it was checked that the surfactant solutions without any additional alcohol do not show a significant enzymatic activity.[27] Even at surfactant concentrations of 22 mass %, the activity remained clearly under 5% of the activity of a 10^{-2} M ethanol solution. This result proved that the oxidation of the surfactant can be neglected in a first approximation.

In a second test, the relative HLADH activities, A, were measured in binary buffer/alcohol media (i.e., without surfactant) as a function of the ratio between the amount of substrate (alcohol, in mole) and the mass of water (in gram), $R_{a/w}$, see Figures 3 and 4. For 1-nonanol (C_9OH) and 1-decanol ($C_{10}OH$), the solubility was too low to perform valuable experiments without adding surfactant.

For the series ethanol to 1-hexanol, the maximum activities, A_{max}, of the enzyme were all between 1.2 and 1.6 without a noticeable trend. However, the substrate concentration, c_{max}, at which these activities occurred decreased with increasing chain length from about 10^{-4} mol of ethanol per gram of buffer to 5×10^{-7} mol of 1-hexanol per gram of buffer.

The diol systems showed a different behavior. 1,5-Pentanediol had the highest A_{max} value and also the highest c_{max} value. The corresponding A_{max} of 1,7-heptanediol was also high, and c_{max} was comparable to the c_{max} value of the 1-pentanol system.

3.3. Activity Dependence on Alcohol Concentration for Different Surfactant Concentrations. In Figures 5−10, the enzymatic activities, A, in the different ternary systems, water/alcohol/$C_{12}E_{23}$, are shown for different surfactant concentrations. For the ethanol system, A decreased with increasing $C_{12}E_{23}$ concentration and neither the overall slope of the curves nor c_{max} were significantly altered by the presence of the surfactant. For the 1-propanol system, similar curves were obtained for the different surfactant concentrations.

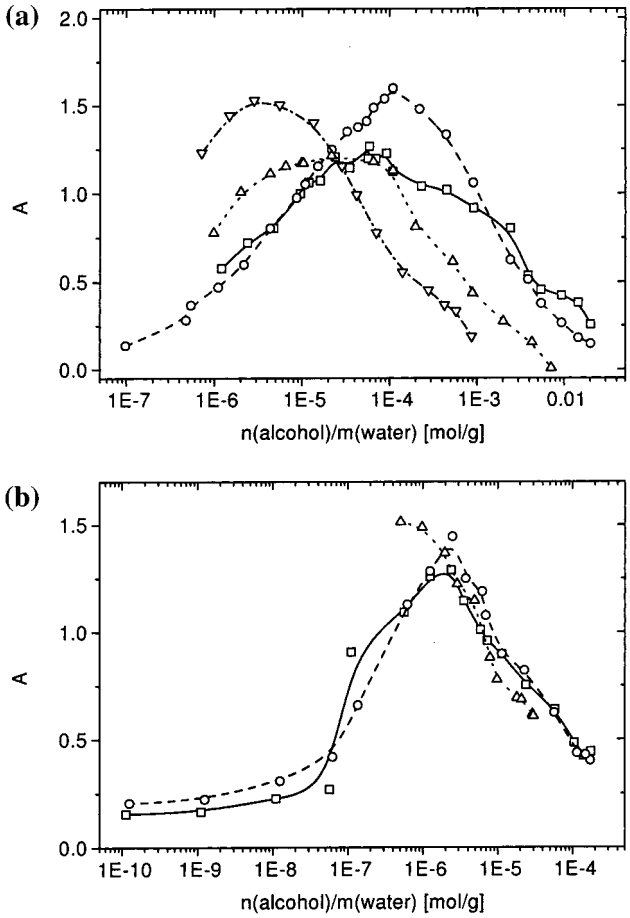

Figure 3. Enzyme activities in binary systems without surfactant. Panel a shows the results for systems containing ethanol (□, batch A of HLADH, and ○, batch B, as defined in section 2.1), 1-propanol (△, batch B), and 1-butanol (▽, batch B). Panel b shows the results for systems containing 1-pentanol (□, batch A of HLADH, and ○, batch B) and 1-hexanol (△, batch B). The different curves are spline fits to guide the eyes.

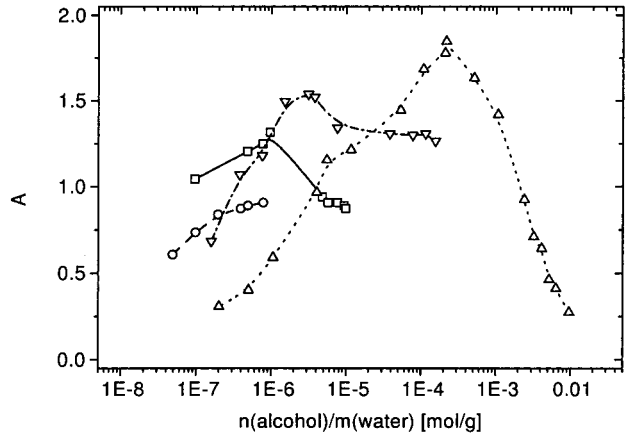

Figure 4. Enzyme activities in binary systems without surfactant. The results are shown for systems containing 1-heptanol (□), 1-octanol (○), 1,5-pentanediol (△), and 1,7-heptanediol (▽) (batch B of HLADH in all four cases). The different curves are spline fits to guide the eyes.

In the series of the ternary 1-butanol to 1-octanol systems, c_{max} was more and more shifted to higher values with increasing

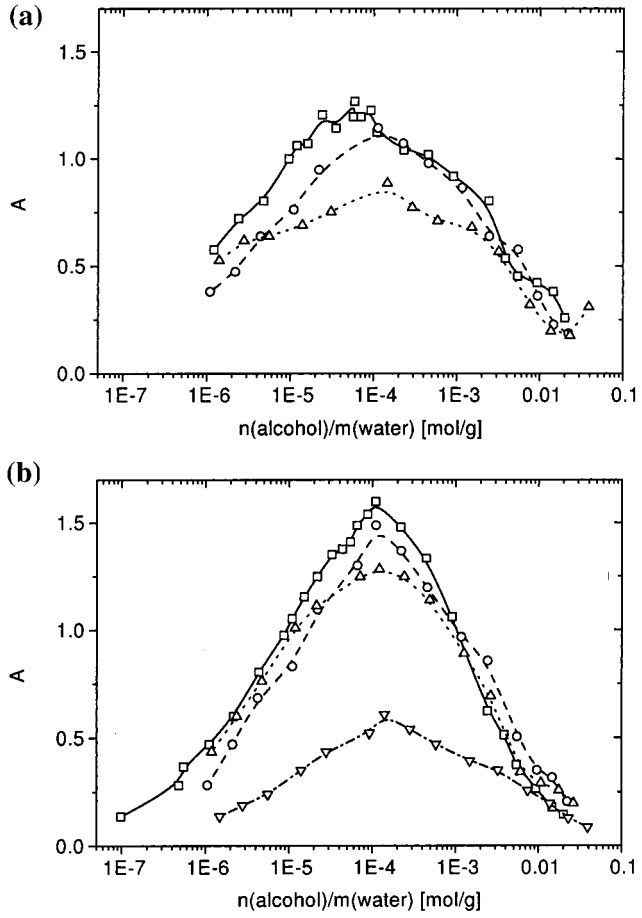

Figure 5. Enzyme activities in ternary $C_{12}E_{23}$/alcohol/water systems. Panel a shows the results for systems containing ethanol as alcohol: (□) 0% of $C_{12}E_{23}$, (○) 1% of $C_{12}E_{23}$, and (△) 22% of $C_{12}E_{23}$; batch A of HLADH in all three cases. Panel b shows the results for systems containing ethanol as alcohol: (□) 0% of $C_{12}E_{23}$, (○) 1% of $C_{12}E_{23}$, (△) 8% of $C_{12}E_{23}$, and (▽) 22% of $C_{12}E_{23}$; batch B of HLADH in all four cases. The different curves are spline fits to guide the eyes.

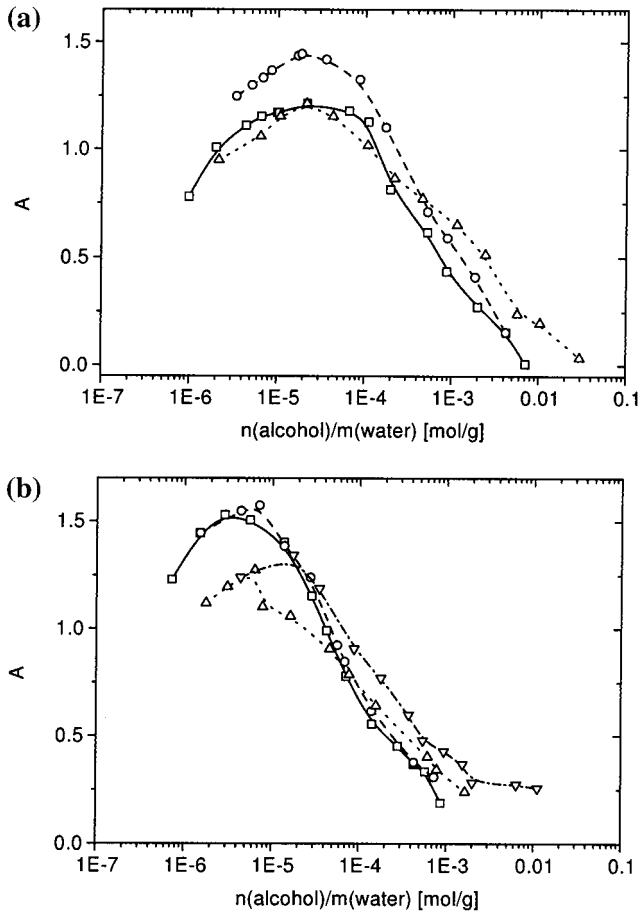

Figure 6. Enzyme activities in ternary $C_{12}E_{23}$/alcohol/water systems. Panel a shows the results for systems containing 1-propanol: (□) 0% of $C_{12}E_{23}$, (○) 1% of $C_{12}E_{23}$, and (△) 22% of $C_{12}E_{23}$; batch B of HLADH in all three cases. Panel b shows the results for systems containing 1-butanol as alcohol: (□) 0% of $C_{12}E_{23}$, (○) 1% of $C_{12}E_{23}$, (△) 8% of $C_{12}E_{23}$, and (▽) 22% of $C_{12}E_{23}$; batch B of HLADH in all four cases. The different curves are spline fits to guide the eyes.

alcohol chain length and increasing surfactant concentration. For C_8OH, A_{max} was even comparable for widely different surfactant concentrations.

The alcohols with the longest hydrocarbon chain, C_9OH and $C_{10}OH$, are unsoluble in pure water, but at the two given surfactant concentrations, the shape of the curves and their shifts to higher alcohol concentrations fitted in the series of the other alcohols.

Concerning the used diols, we found that, in our systems, 1,5-pentanediol behaves like ethanol and 1-propanol. Even the c_{max} values of 1,5-pentanediol and ethanol were comparable. However, in contrast to the ethanol system, the absolute enzyme activity values, A, were nearly independent of the surfactant concentration, just as it was for the 1-propanol system. 1,7-Heptanediol had a much lower c_{max} value and behaved similarly to 1-pentanol.

4. Discussion

4.1. Phase Diagrams. The influence of the various alcohols on the extent of the realms of existence of monophasic, isotropic, and thermodynamically stable liquids of low viscosity has already been discussed in previous papers.[2,3] In particular, it could be shown that the alcohols with at least four carbon atoms can play the role of a cosurfactant. In contrast, the short alcohols do not participate in the structuring of micelles, and at least in the case of ethanol, the alcohol molecules even prevent the formation of micelles so that true molecular solutions are formed at high alcohol concentrations.[28,29]

4.2. Preliminary Tests. All activity curves measured in binary buffer/alcohol media show the expected behavior, as it is sketched in Figure 11: in the dilute "Michaelis−Menten regime", the activity increases and reaches a maximum initial velocity, V_{max}. The dependence of the initial velocity on the substrate concentration can be described by the following equation, the so-called Michaelis−Menten equation:[30,31]

$$V = \frac{V_{max}[S]}{K_M + [S]} \quad (1)$$

where [S] denotes the substrate concentration and K_M the Michaelis constant.

For higher "non-Michaelis" concentrations, the enzyme is inhibited by the substrate and consequently the enzymatic activity decreases. This behavior can be explained by assuming *uncompetitive* inhibition of the enzyme by the substrate. In this

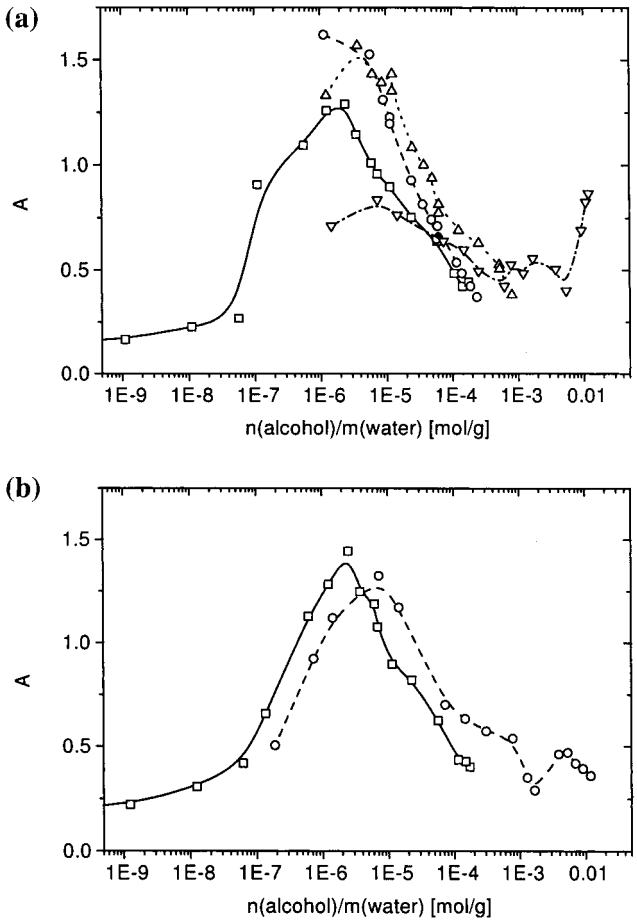

Figure 7. Enzyme activities in ternary $C_{12}E_{23}$/alcohol/water systems. Panel a shows the results for systems containing 1-pentanol: (□) 0% of $C_{12}E_{23}$, (○) 1% of $C_{12}E_{23}$, (△) 8% of $C_{12}E_{23}$, and (▽) 22% of $C_{12}E_{23}$; batch A of HLADH in all four cases. Panel b shows the results for systems containing 1-pentanol: (□) 0% of $C_{12}E_{23}$ and (○) 22% of $C_{12}E_{23}$; batch B of HLADH in both cases. The different curves are spline fits to guide the eyes.

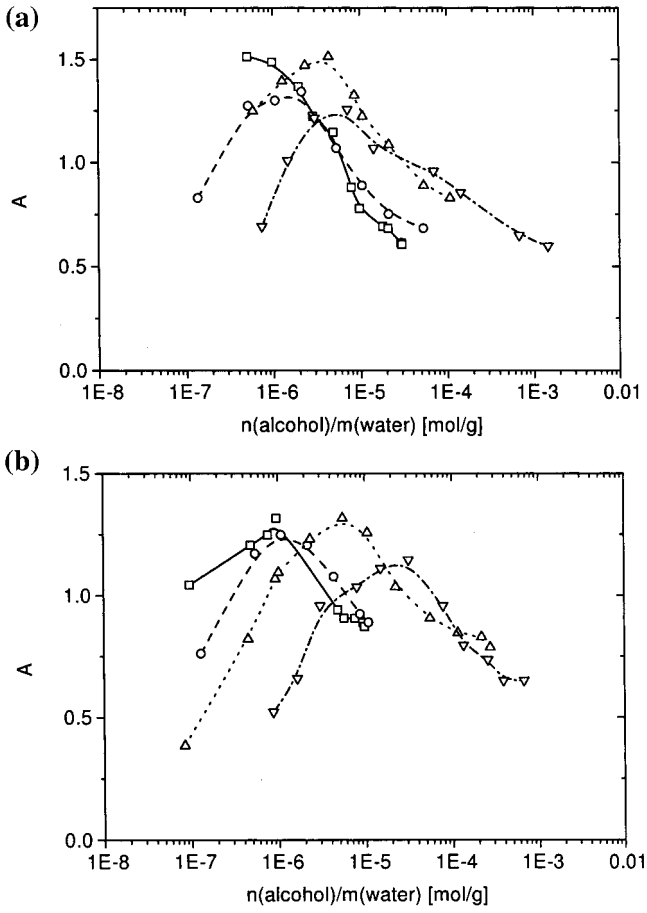

Figure 8. Enzyme activities in ternary $C_{12}E_{23}$/alcohol/water systems. Panel a shows the results for systems containing 1-hexanol as alcohol: (□) 0% of $C_{12}E_{23}$, (○) 1% of $C_{12}E_{23}$, (△) 8% of $C_{12}E_{23}$, and (▽) 22% of $C_{12}E_{23}$; batch B of HLADH in all four cases. Panel b shows the results for systems containing 1-heptanol: (□) 0% of $C_{12}E_{23}$, (○) 1% of $C_{12}E_{23}$, (△) 8% of $C_{12}E_{23}$, and (▽) 22% of $C_{12}E_{23}$; batch B of HLADH in all four cases. The different curves are spline fits to guide the eyes.

case, following reaction scheme is appropriate:

$$\begin{array}{c} \mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \\ + \mathrm{I} \\ k_4 \uparrow\downarrow k_{-4} \\ \mathrm{ESI} \end{array} \tag{2}$$

where E and P denote the enzyme and the product, respectively, and k_i denotes the velocity constants. With

$$K_\mathrm{M} = \frac{k_{-1} + k_2}{k_1} \tag{3}$$

and

$$K_\mathrm{I} = \frac{k_{-4}}{k_4} \tag{4}$$

the following theoretical expression is obtained:

$$V = \frac{V_{\max}[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}] + \dfrac{[\mathrm{S}][\mathrm{I}]}{K_\mathrm{I}}} \tag{5}$$

where K_I is the Michaelis constant of the inhibitor−enzyme complex and [I] is the inhibitor concentration. In a typical substrate-inhibited reaction, the substrate itself acts as an uncompetitive inhibitor so that [I] = [S]. The corresponding curve is shown in Figure 11.

By contrast, *competitive* inhibition is described by following reaction scheme:

$$\begin{array}{c} \mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \\ + \mathrm{I} \\ k_3 \uparrow\downarrow k_{-3} \\ \mathrm{EI} + \mathrm{S} \end{array} \tag{6}$$

The corresponding equation is

$$V = \frac{V_{\max}[\mathrm{S}]}{K_\mathrm{M}\left(1 + \dfrac{[\mathrm{I}]}{K_\mathrm{I}}\right) + [\mathrm{S}]} \tag{7}$$

where

$$K_\mathrm{I} = \frac{k_{-3}}{k_3} \tag{8}$$

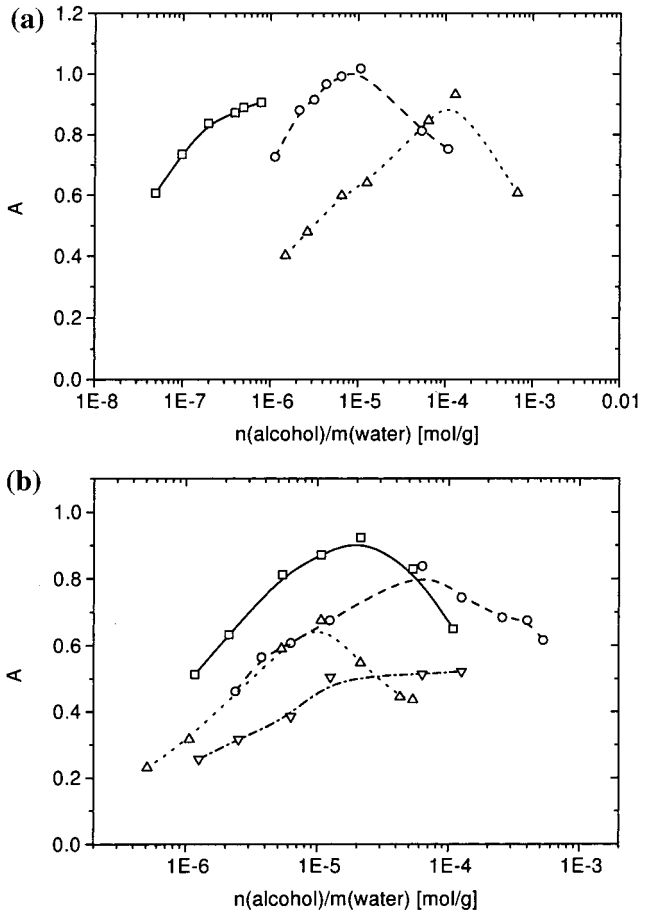

Figure 9. Enzyme activities in ternary $C_{12}E_{23}$/alcohol/water systems. Panel a shows the results for systems containing 1-octanol as alcohol: (□) 0% of $C_{12}E_{23}$, (○) 8% of $C_{12}E_{23}$, and (△) 22% of $C_{12}E_{23}$; batch B of HLADH in all three cases. Panel b shows the results for systems containing 1-nonanol: (□) 8% of $C_{12}E_{23}$, and (○) 22% of $C_{12}E_{23}$; batch B of HLADH in both cases. Panel b also shows results for systems containing 1-decanol: (△) 8% of $C_{12}E_{23}$ and (▽) 22% of $C_{12}E_{23}$; batch B of HLADH in both cases. The different curves are spline fits to guide the eyes.

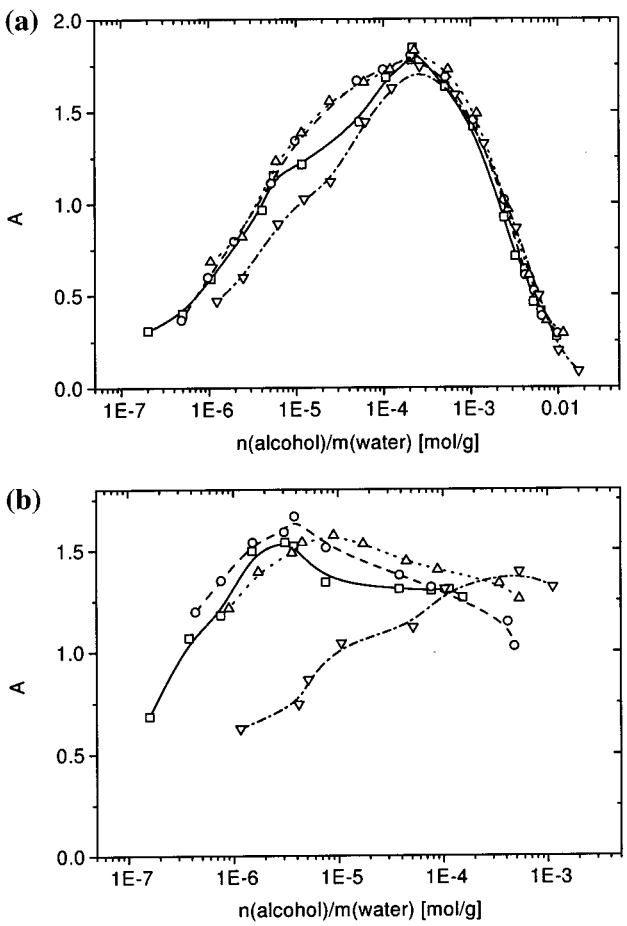

Figure 10. Enzyme activities in ternary $C_{12}E_{23}$/alcohol/water systems. Panel a shows the results for systems containing 1,5-pentanediol as alcohol: (□) 0% of $C_{12}E_{23}$, (○) 1% of $C_{12}E_{23}$, (△) 8% of $C_{12}E_{23}$, and (▽) 22% of $C_{12}E_{23}$; batch B of HLADH in all four cases. Panel b shows the results for systems containing 1,7-heptanediol: (□) 0% of $C_{12}E_{23}$, (○) 1% of $C_{12}E_{23}$, (△) 8% of $C_{12}E_{23}$, and (▽) 22% of $C_{12}E_{23}$; batch B of HLADH in all four cases. The different curves are spline fits to guide the eyes.

As can be seen in Figure 11, this model does not describe correcly the experimental results. Therefore, it can be concluded that in all cases discussed in this paper uncompetitive inhibition occurs.

With long-chain alcohols, the maximum enzymatic activity is obtained already at very low concentrations and the substrate inhibition occurs also already at low concentrations, as shown in section 3.2. This feature suggests that the enzyme has a higher global affinity for long-chain alcohols than for short-chain ones, a result which is in agreement with other studies in aqueous buffer solutions.[9,18] For the long-chain alcohol 1-octanol (C_8-OH), the maximum cannot be reached because of the very limited water solubility range of this mainly hydrophobic alcohol.

In principle, equations similar to those given in this section could be used to analyze the ternary systems. Wang et al.[32] proposed a promising way to do this. However, it is not possible to get independent information about K_M and K_I in our case, and therefore, we do not follow this strategy.

4.3. Activity Dependence on Alcohol Concentration for Different Surfactant Concentrations. In all of our interpretation of the enzymatic activities, we assume that both NAD^+ and HLADH remain in the aqueous pseudophases and do not significantly interact with the interfaces. This assumption is based on a study of HLADH activities in reversed micelles.[11]

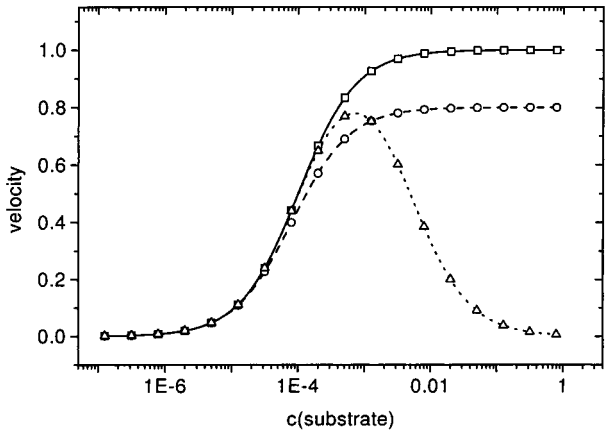

Figure 11. Theoretical behavior of enzyme velocity/activity as function of the substrate concentration: (□) Michaelis−Menten behavior; (○) competitive inhibition; (△) uncompetitive inhibition.

4.3.1. Ternary Systems Containing n-Alkanols. Because ethanol is not incorporated in nanostructures, the behavior of the activity curves shown in Figure 5 is not unexpected. The overall shape of the curves is similar, but the absolute values

decrease with increasing surfactant concentration. One explanation may be a sterical hindrance of the enzyme−substrate interaction by the surfactant molecules, thus reducing A_{max}.

In the case of the 1-propanol system, such a dependence on surfactant concentration is not found, see Figure 6a. The decrease in activity due to the presence of single surfactant molecules may be balanced by specific interactions between surfactant and alcohol molecules. Although 1-propanol molecules are certainly not yet incorporated into micelles—the solubility of 1-propanol in water is infinite—some hydrophobic interactions between $C_{12}E_{23}$ and 1-propanol may occur with the consequence that less alcohol molecules come into contact with the enzyme molecules.

Concerning the series of the 1-butanol to 1-decanol ternary system, the influence of the stabilizing effect of the surfactant on the enzyme activity, as shown in Figures 6−9, becomes more and more apparent. For the ternary systems containing the alcohols C_nOH with $n = 4-7$, the partition coefficient, p, of the alcohol was estimated from the modeling of the corresponding SANS spectra and, in one case, of the self-diffusion coefficients.[3] The coefficient p is the mass fraction of alcohol inside the micelles:

$$p_{C_nOH} = \frac{m_{C_nOH}^{(micelle)}}{m_{C_nOH}^{(micelle)} + m_{C_nOH}^{(bulk)}} \tag{9}$$

where $m_{C_nOH}^{(micelle)}$ and $m_{C_nOH}^{(bulk)}$ denote the mass of alcohol associated with or incorporated in the micelles and in the continuous outer pseudophase, respectively. The following values have been found: $p_{C_4OH} = 0.15$, $p_{C_5OH} = 0.38$, $p_{C_6OH} = 0.9$, and $p_{C_7OH} = 0.99$ for surfactant concentrations of 8 mass %.

Now let us see if this trend can be at least qualitatively inferred from the enzymatic activity. We suppose that the c_{max} values of a given alcohol system, but for different $C_{12}E_{23}$ concentrations, roughly correspond to the same effective alcohol concentration, c_{eff}, in the aqueous pseudophase. This effective alcohol concentration should roughly be the same as the measured c_{max} value at zero surfactant concentration, c_{max}^0. Within this hypothesis, the concentration of alcohols in the micelles, $c_{C_nOH}^{(micelle)}$, is given by

$$c_{C_nOH}^{(micelle)} = c_{max} - c_{eff} = c_{max} - c_{max}^0 \tag{10}$$

Then we can estimate the alcohol partition coefficient, p, as

$$p_{C_nOH} = \frac{c_{max} - c_{max}^0}{c_{max}} \tag{11}$$

From Figures 3−10 following p values can be roughly inferred: $p_{C_4OH} = 0.5$, $p_{C_5OH} = 0.6$, $p_{C_6OH} = 0.9$, and $p_{C_7OH} = 0.9$ at 8 mass % of $C_{12}E_{23}$.

Apart from the value for the butanol system, the other p values are in reasonable agreement with those inferred from the SANS measurements. Taking into account the considerable scattering of the enzymatic activity data, the result is very satisfactory. Probably, more data around the c_{max} values would further improve the agreement.

For the highest surfactant concentration systems (22 mass % of $C_{12}E_{23}$), no modeling of the SANS data was possible and consequently there are no reference data for the alcohol partition coefficients. The enzyme activity measurements give following p values for this $C_{12}E_{23}$ concentration according to eq 11: $p_{C_2OH} = 0$, $p_{C_3OH} = 0$, $p_{C_4OH} = 0.8$, $p_{C_5OH} = 0.7$, $p_{C_6OH} = 0.9$, $p_{C_7OH} = 1$, and $p_{C_8OH} = 1$.

So, it seems that with increasing amounts of structuring material the partition coefficients are increasing whereas the shape of the nanostructures themselves (spherical, rodlike micelles, ...) is of minor importance. Two other observations should be mentioned: the enzymatic activity, A_{max}, of long-chain alcohols is lower than A_{max} of short-chain alcohols. Although the long-chain alcohols have a higher affinity for the enzyme, see section 3.2, they are not as efficiently oxidized by the enzyme as short-chain alcohols. This is in keeping with the fact that $C_{12}E_{23}$, a very long-chain alcohol, is only poorly attacked by the enzyme. Therefore, we conclude that the high affinity of the enzyme for long-chain alcohols does not correspond with a high catalytic efficiency. It is probable that substrate inhibition occurs even for substrate concentrations $c < c_{max}$, that is, for concentration ranges in which a Michaelis−Menten mechanism can be considered to describe the enzyme kinetics.

Furthermore, the highest surfactant concentration prevents the enzyme activity from going to zero in the case of 1-pentanol solutions. This point was already discussed in a previous paper.[2] For the present paper, we repeated the corresponding experiments and we extended them to very high alcohol concentrations. Thus, we not only confirmed the previous results, but we also discovered a strong and reproducible increase in A at high substrate concentrations. Probably, the partition coefficient varies with the alcohol concentration so that the effective alcohol concentration in the aqueous pseudophase is more favorable at high total alcohol concentrations in the system.

4.3.2. Ternary Systems Containing α,ω-Alkanediols. As mentioned in the Introduction, diols were proposed in the literature for the formulation of nontoxic microemulsions, especially by Kahlweit's group.[6−8] They were mainly concerned with 1,2-alkanediols, which are much more surface-active than α,ω-alkanediols.[8,33] Alany et al.[5] studied 1,2-diols in ethyl oleate/nonionic surfactant/water systems, and they found no correlation between the cosurfactant or cosolvent behavior of 1,2-diols and *n*-alcohols except in the case of 1,2-hexanediol, which behaves somewhat similarly to 1-butanol. These authors argued that their results are in contrast to what was found by Kahlweit,[6−8] and they concluded that any generalization of alcohols and diols depends also on the oil and surfactant combination used.

Cañadas et al.[4] studied the behavior of 1,6-hexanediol in the presence of hexadecyltrimethylammonium bromide (C_{16}TAB) and Karukstis et al.[34] studied that of 1,5-pentanediol and 1,7-heptanediol in the presence of different alkyltrimethylammonium bromides. Both groups stated that α,ω-alkanediols should act as cosolvents, similar to short-chain alcohols. Because of their end-standing hydroxyl groups, an insertion of α,ω-diols into the micelle interface should thermodynamically be unfavorable.

In our experiments, 1,5-pentanediol behaves like short-chain *n*-alkanols, see Figures 5, 6a, and 10a. All of the diol molecules are in the aqueous pseudophase or on the surface of the micelles and therefore accessible to the enzyme molecules. Within the precision of the experiments, the partition coefficients for 1,5-pentanediol are $p(8\%\ C_{12}E_{23}) = 0$ and $p(22\%\ C_{12}E_{23}) = 0.3$. Furthermore, the phase diagram of 1,5-pentanediol is similar to those found for corresponding short-chain alcohols.[35]

Concerning 1,7-heptanediol, the much lower c_{max} value obtained from Figure 10b results in a higher affinity of HLADH to this alcohol, as is the case for long-chain alcohols. For 1,7-

heptanediol, the partition coefficients are $p(8\%\ C_{12}E_{23}) = 0.7$ and $p(22\%\ C_{12}E_{23}) = 1$.

This diol best compares with 1-pentanol, but the incorporation of the diol into the micellar pseudophase is apparently still more important at high surfactant concentrations. This is in contrast to the arguments in the literature[34,36] in which it is claimed that diols solubilize on the micellar surface because they have steric difficulties for solubility in the micellar interior. This argument seems only to be true for diols with alkyl chains shorter than that of 1,7-heptanediol. Note that 1,7-heptanediol is not miscible in all proportions with water.

4.3.3. Comparison of n-Alkanols and **α,ω-Alkanediols.** As a conclusion of our enzyme activity studies over large alcohol concentration ranges, we observe a clear difference between short-chain alcohols (ethanol, 1-propanol, 1,5-pentanediol) acting as a cosolvent of water and longer-chain alcohols, which have a significant affinity for the micellar pseudophase and which can therefore be considered as cosurfactants or at least as coaggregates.[34]

5. Conclusion

Enzyme activities can be very sensitive to the nanostructure of complex liquids. In some cases, the measurement of such activities are a valuable complement to optical probes. In particular, the cosolvent, cosurfactant, or coaggregate role of alcohols can be investigated in detail. A further advantage is the simple experimental equipment required, because activities of some enzymes such as HLADH can be easily recorded with standard spectrometers. Finally, this strategy can also be interesting in biotechnology, because enzymatic reactions can be optimized by a convenient tuning of the nanostructured reaction medium.

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