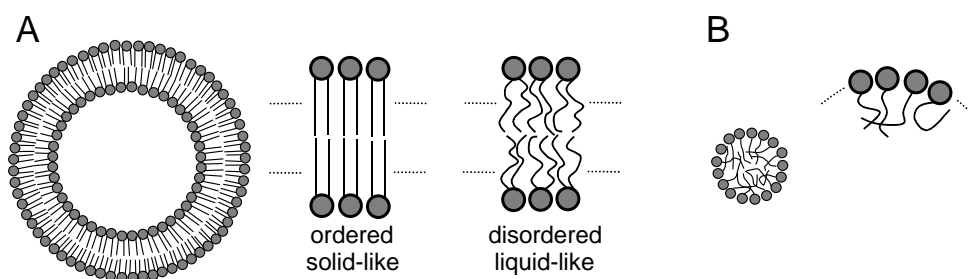


SUMMARY

Vesicular catalysis can be an important tool in understanding non-enzymatic catalysis in biological membranes. So far, most studies involving vesicular catalysis have focused on differences compared to micellar catalysis. As discussed in Chapter 1, these differences mainly result from the enclosure of an aqueous compartment by a hydrophobic bilayer of amphiphiles in vesicles (Scheme 1A), whereas micelles can be regarded as oil-like droplets (Scheme 1B). As a consequence, the inner and outer leaflet of the bilayer might be differentiated kinetically if permeation of reactants is relatively slow. In addition, the tails of the double-tailed amphiphiles can be in a more rigidly ordered (gel-like) phase or a more fluidly, disordered (liquid-crystalline) phase, whereas in micelles the tails are always in a fluid-like phase (Scheme 2). Due to differences in packing efficiency, micelles have a more “open” structure leading to a higher water concentration at the polar-apolar interface.

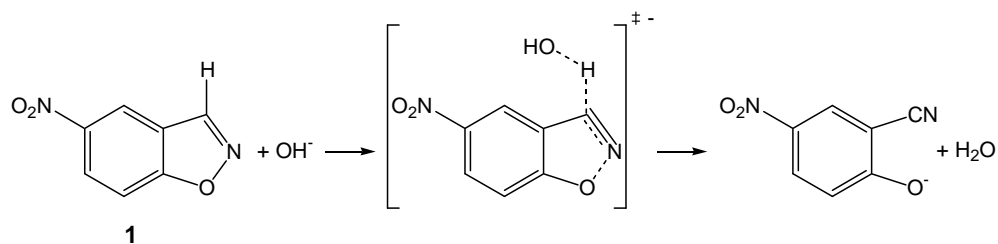


Scheme 1. Schematic representation of a vesicle formed from double-tailed surfactants with tails in either the ordered or disordered phase (A) and a micelle formed from single-tailed surfactants (B).

As is evident from the extensive literature reports on catalysis by micelles formed in the presence of (large) hydrophobic additives, this topic has been well studied for a wide variety of reactions and systems. Only few of such studies are known when it comes to vesicular catalysis.

It should be noted that biological membranes are complex mixtures of a large variety of compounds. In addition, these compounds have a number of functional groups. Therefore, they can undergo several interactions with their environment leading to a complex interplay of interactions.

Considering that vesicular catalysis has mainly been performed in single-component systems and that biological membranes are complex mixtures of a variety of compounds there is a void in the understanding of catalysis at the interface of biological membranes. The influence of the addition of four different classes of additives on the vesicular catalysis of a model reaction has been explored in this thesis. The amphiphile chosen is dimethyldi-*n*-octadecylammonium chloride ($\text{C}_{18}\text{C}_{18}^+$). The four classes of additives are anionic double-tailed amphiphiles, long linear *n*-alcohols, ethylene-glycol surfactants and *n*-dodecyl- β -pyranosides. As a suitable model reaction the hydroxide-ion catalysed deprotonation reaction of 5-nitrobenzoxazole (**1**; Scheme 2) was chosen. Not only is the rate constant sensitive to the local hydroxide-ion concentration at the vesicular binding sites, but also to the local polarity and the extent of dehydration of the reactants upon binding to the vesicular binding sites. In particular, dehydration of hydroxide ions can lead to large rate accelerations.



Scheme 2. Kemp elimination reaction of 5-nitrobenzisoxazole by hydroxide ions.

Before addressing catalytic effects modified by the addition of the four classes of additives, the overall properties of the vesicles formed in the presence of the additives are discussed in Chapter 2. The main phase transition temperatures (T_m), the temperature at which the tails go from the gel-like to the liquid-crystalline phase, is lowered in the presence of all additives at a low mole fraction (<10 mol%). At higher mole fractions, the T_m decreases further for the additives that are, in principle, single-tailed surfactants: *n*-dodecyl- β -glucoside (**C₁₂Glu**), *n*-dodecyl- β -maltoside (**C₁₂Mal**), and eicosa-ethylene glycol mono *n*-hexadecyl ether (**C₁₆EO₂₀**). For the saturated alcohols the T_m increases again, and the extent of the increase follows the trend *n*-decanol (**C₁₀OH**) < batyl alcohol (**C₁₈GOH**) < *n*-octadecanol (**C₁₈OH**). The mole fraction, at which the increase in T_m starts, follows the reverse order. Upon increasing the amounts of oleyl alcohol (**C_{18:1}OH**) the transition becomes progressively less cooperative. In the case of the anionic amphiphiles, sodium di-*n*-decylphosphate (**C₁₀C₁₀⁻**) and sodium *n*-decyl-*n*-octadecylphosphate (**C₁₀C₁₈⁻**), (neutral) microdomains are formed between 5 mol% and 35 mol%. However, the vesicular catalysis was in all cases studied at 15°C, which is above the T_m of vesicles formed in the presence of the additives.

Upon addition of **C₁₂Mal** and **C₁₆EO₂₀** vesicles are partially solubilised into mixed micelles. As is determined by both dynamic light scattering and turbidity experiments, the extent of solubilisation depends on both the mole fraction of single-tailed surfactant and the concentration of **C₁₈C₁₈⁺**.

Five different dyes, pyrene, the $E_T(30)$ -dye, laurdan, Nile Red and 1,8-ANS, were used to address changes in the polarity of the vesicular surface. These dyes have been employed in the literature to report the polarity in solvents and solvent mixtures. They were selected on the basis of the different sensitivity towards interactions with their environment. For example, Nile Red and the $E_T(30)$ -dye are particularly sensitive towards hydrogen-bond donation, whereas pyrene is not. Despite these differences in sensitivity no significant change in membrane polarity is observed upon addition of the additives.

In Chapter 3 it is shown that in 1,4-dioxane-water and acetonitrile-water mixtures the natural logarithm of the rate constant for the Kemp elimination increases linearly with the polarity as reported by Nile Red. The increase in rate constant mainly originates from dehydration of the hydroxide ion, leading to destabilisation of the initial state relative to the activated complex.

In a typical experiment solely in the presence of cationic vesicles, there is initially a sharp increase of the observed rate constant (k_{obs}) to a maximum value upon increasing the amphiphile concentration. Then k_{obs} slowly decreases. The *observed catalysis*, expressed as the ratio of the maximum observed rate constant and the observed aqueous rate constant ($k_{\text{obs,max}}/k_{\text{w,obs}}$), amounts to a factor of *ca.* 1000. This type of behaviour is characteristic for

vesicular catalysis of bimolecular reactions and can be described by the pseudophase model with ion exchange derived in the late 1970s. This model assumes two pseudophases, an aqueous one and a vesicular one. Two effects contribute to the observed catalysis. The first effect comes from a decreased effective reaction volume, since both reactants are efficiently bound to the vesicular pseudophase. Hence, the local concentration of reactants is much higher than in the bulk solution. The second effect originates from an increase in rate constant going from the aqueous pseudophase to the vesicular pseudophase mainly as a result of partial dehydration of the reactants, similar as for the water-organic solvent mixtures. Taking these effects into account the *catalytic rate acceleration*, defined as the ratio of the bimolecular vesicular and aqueous rate constants ($k_{\text{ves}}/k_{\text{w}}$), amounts to a factor of *ca.* 50.

The parameters that can be obtained by fitting the observed rate constant as a function of the amphiphile concentration by the pseudophase model are (i) the vesicular rate constant (k_{ves}), (ii) the binding constant of **1** (K_{S}), (iii) the counterion binding (β) and (iv) the ion exchange constant ($K_{\text{OH}}^{\text{Cl}}$). A detailed error analysis revealed that the parameters can compensate each other to a large extent. However, the ion exchange constant can be estimated independently and it was found that this parameter does not vary upon addition of the anionic amphiphiles. Allowing the other three parameters to vary simultaneously leads to erroneous results, such as a counterion binding that is larger than unity. Therefore, at most only two parameters were allowed to vary. As is apparent from the kinetic equation, compensation of k_{ves} and K_{S} is particularly large when the binding constant is small.

Based on the observation that the polarity does not change upon addition of the anionic amphiphiles $\text{C}_{10}\text{C}_{10}^-$ and $\text{C}_{10}\text{C}_{18}^-$ to vesicles formed from $\text{C}_{18}\text{C}_{18}^+$, it is anticipated that the decrease in observed rate constant upon increasing amounts of $\text{C}_{10}\text{C}_{10}^-$ or $\text{C}_{10}\text{C}_{18}^-$ is due to a reduced counterion binding. Not only acts the anionic head group as a counterion, but also the formed neutral pairs dilute the remaining cationic head groups in the vesicle leading to a lower positive surface charge density. Hence, unfavourable head group-head group interactions are reduced and less counterion binding is required to reduce these interactions. Calculation of the charge per amphiphile (which is a measure of the surface charge density) from the counterion binding percentages reveals that the overall charge per amphiphile does not vary significantly upon the addition of $\text{C}_{10}\text{C}_{10}^-$ or $\text{C}_{10}\text{C}_{18}^-$ in concentrations up to 35 mol%.

In Chapter 4 it is reported that addition of 50 mol% of C_{10}OH to vesicles formed from $\text{C}_{18}\text{C}_{18}^+$ only leads to a decrease of 10% in the maximum observed rate constants, whereas addition of 50 mol% of C_{18}GOH and C_{18}OH leads to decreases by 40% and 60%, respectively. On the contrary, addition of 35 mol% of $\text{C}_{18:1}\text{OH}$ leads to an *increase* by 75% in the maximum observed rate constant. At higher mole fractions the observed rate constant decreases again. It is discussed that solely a decrease in counterion binding cannot account for the change in observed rate constants, since (i) each alcohol would then have their own specific ability to reduce the counterion binding and (ii) addition of $\text{C}_{18:1}\text{OH}$ would lead to an *increase* in counterion binding above unity. From the literature data on charged micelles containing neutral additives, it is found that the counterion binding decreases rather irrespective of the structure of the additive. Therefore, the experimental data was fitted using a decrease in counterion binding irrespective of the structure of the

alcohol based on the results obtained for **C₁₀C₁₀⁻**. It is assumed that four alcohol molecules require the same area as one (neutral) pair of **C₁₀C₁₀⁻** and **C₁₈C₁₈⁺**. Then, the rest of the increase or decrease in observed rate constants was attributed to a change in k_{ves} and/or K_{S} , since the ion-exchange constant does not depend on the structure or mole fraction of the additive. A detailed interpretation of the results is hampered by the small effects (typically smaller than a factor of four), but overall it appears to involve a subtle interplay. Chapter 5 describes the influence of the presence of (oligo) ethylene glycol units in the Stern region of cationic vesicles. Addition of small amounts of **C₁₆EO₂₀** (≤ 10 mol%) to vesicles formed from **C₁₈C₁₈⁺** does not lead to any change in the observed rate constants. At 35 mol% of **C₁₆EO₂₀** the maximum observed rate constant has decreased by about 30% as a result of micelle formation. This difference in catalytic efficiency going from vesicles to micelles is confirmed by literature experiments using the single-tailed surfactant *n*-octadecyltrimethylammonium chloride (**C₁₈⁺**).

Chapter 6 reports the 7- and 4.5-fold increase in the maximum observed rate constants upon addition of 50 mol% of **C₁₂Glu** and **C₁₂Mal**, respectively, to vesicles formed from **C₁₈C₁₈⁺**. The origin of this large effect comes from a more extensive dehydration of the reactants, due to replacement of water molecules from the Stern region by the sugar moieties. It is discussed that specific or preferential binding of hydroxide ions cannot be the origin of the observed increase of the rate constant. Deprotonation of the hydroxyl groups by the vesicle-bound hydroxide ions of the pyranosides might account for the observed catalytic effects as well, although it appears to be unlikely. Analysis of the experimental data reveals that the increase in the maximum observed rate constant mainly originates from an increase in k_{ves} for **C₁₂Glu**, whereas it comes mainly from an increase in K_{S} for **C₁₂Mal**. Due to micelle formation upon the addition of **C₁₂Mal**, k_{ves} decreases after an initial increase.

Chapter 7 puts the results of the previous chapters into perspective. At the end of the chapter some possibilities for future research are given.

