Vesicular catalysis of the decarboxylation of 6-nitrobenzisoxazole-3-carboxylate. The effects of sugars, long-tailed sugars, cholesterol and alcohol additives

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ABSTRACT: The effects of the addition of sugars, long-tailed *n*-alkyl pyranosides, *n*-alkyl glycerol ethers and *n*-alcohols on the properties of di-*n*-hexadecyldimethylammonium bromide (DHAB) vesicles have been studied. Properties that were examined include the stability, morphology, phase of the tails, and catalytic rate acceleration of the unimolecular decarboxylation of the 6-nitrobenzisoxazole-3-carboxylate anion (6-NBIC). The kinetic data were analyzed on the basis of the pseudophase model and show a rate acceleration of a factor of about 1000 relative to the reaction in water. Upon addition of most additives an inhibiting effect on the decarboxylation reaction of 6-NBIC is observed relative to the reaction in vesicles without any additive. The largest inhibition was observed in the case of cholesterol. Contrastingly, *n*-dodecyl-*β*-maltoside (in the spherical vesicle region) and trehalose accelerate the reaction. The activation parameters show that the most significant contribution to the Gibbs energy of activation is the enthalpic factor, with a partly compensating entropic contribution. Copyright © 2006 John Wiley & Sons, Ltd.
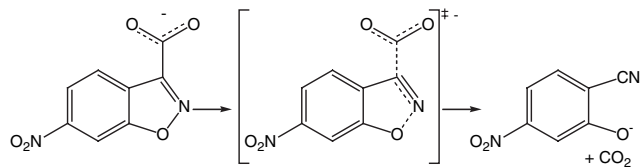
KEYWORDS: 6-NBIC; DHAB; vesicles; vesicular catalysis

INTRODUCTION

The membrane is an essential part of the biological cell by aiding replication, compartmentalizing the cell contents and controlling the flux of small molecules into and out of the cell.[1] Vesicles formed from (mixtures of) phospholipids and/or synthetic amphiphiles have been used as mimics for such membranes. Due to the often complex structure of biomolecules, little is known about interactions on a molecular scale between the various components of biological membranes.[2] Previously, reaction kinetic measurements of bimolecular reactions have been employed in attempts to understand some of these interactions in model membranes composed of more than one amphiphilic component. In these systems, besides one or more types of amphiphile (which form vesicles on their own), other amphiphilic molecules such as long-tailed alcohols and *n*-alkyl pyranosides were added in various concentrations. It was found that the direction and extent of the effect on the vesicular rate constant and the binding constant of the organic substrate upon the addition of long-tailed alcohols depend strongly on the nature of the alkyl tail. In the case of the addition of *n*-alkyl pyranosides it was concluded that partial dehydration of small ions present at the interface takes place. Concomitantly, the local water concentration is reduced. However, for bimolecular reactions the reactivity and distribution of both reactants have to be considered, often leading to difficulties in interpretation of the experimental data. Alternatively, when a unimolecular reaction is used these problems do not play a role, therefore the unimolecular decarboxylation reaction of the 6-nitrobenzisoxazole-3-carboxylate anion (6-NBIC, Scheme 1) was selected as a suitable reaction, because this reaction is also very sensitive to changes in the local polarity at the binding site(s) of the reactant.[3–6] Apolar solvents result in the formation of ion pairs, thereby reducing the rate constant for the decarboxylation reaction. Increasing the polarity will therefore increase the rate constant compared to apolar solvents. Hydrogen bonding, however, leads to a decrease in the rate constant because of dominant hydrogen bond stabilization of the initial state. Cationic vesicles accelerate this decarboxylation reaction relative to pure water by a factor of ca. 1000.[6] It has been shown that such vesicles can accelerate the 6-NBIC reaction by approximately 30% compared to cationic micelles.[7] Synthetic cationic vesicles are also 4–15 times more efficient for the decarboxylation than phospholipid vesicles.[8] The interaction between the

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Scheme 1. Decarboxylation reaction of 6-nitrobenzisoxazole-3-carboxylate (6-NBIC)

hexadecanol (HD). Class B contains two glycerol ethers (with one or two alkyl tails) and cholesterol. Class C includes C_{12}-tailed surfactants with sugar headgroups. Class D contains sugars without a hydrophobic anchor: glucose (Glu), maltose (Mal) and trehalose (Treh). In addition to kinetic measurements, differential scanning calorimetry (DSC) and cryo-transmission electron microscopy (cryo-TEM) measurements have been carried out.

cationic head group and the 6-NBIC anion promotes binding at the vesicular interface, thereby decreasing the number of hydrogen bonding opportunities between water and the substrate, with a concomitant increase of the rate constant. It is thus expected that the additives will affect the local environment of the substrate binding sites, resulting in changes in the vesicular rate constants.

Di-*n*-hexadecyldimethylammonium bromide (DHAB) was selected as the vesicle-forming amphiphile. Four classes of additives (Scheme 2) were chosen, with systematic changes in their structure in order to gain an understanding of the effect of each component on the unimolecular decarboxylation reaction. Class A includes the monohydric alcohols *n*-dodecanol (DD) and *n*-

EXPERIMENTAL

Di-*n*-hexadecyldimethylammonium bromide (DHAB $\geq$ 97%), *n*-hexadecanol ($\geq$99%) and *n*-dodecyl-β-D-glucopyranoside ($\geq$99%) (CMC $= 1.9 \times 10^{-4}$ mol l^{-1})[9] were obtained from Fluka. Cholesterol ($\geq$99%) was purchased from Aldrich, acetonitrile (99%) was from Acros, D(+)-glucose (anhydrous) was bought from Merck and maltose from Sigma. 1-O-*n*-Hexadecyl-rac-glycerol and 1,2-O-di-*n*-hexadecyl-rac-glycerol were purchased from Brunschwig Chemie and stored at -20°C. *n*-Dodecyl-β-maltoside (>99.5%) (CMC $= 1.5 \times 10^{-4}$ mol l^{-1})[9] was purchased from Glycon and was also stored at -20°C. All chemicals were used without further purification.
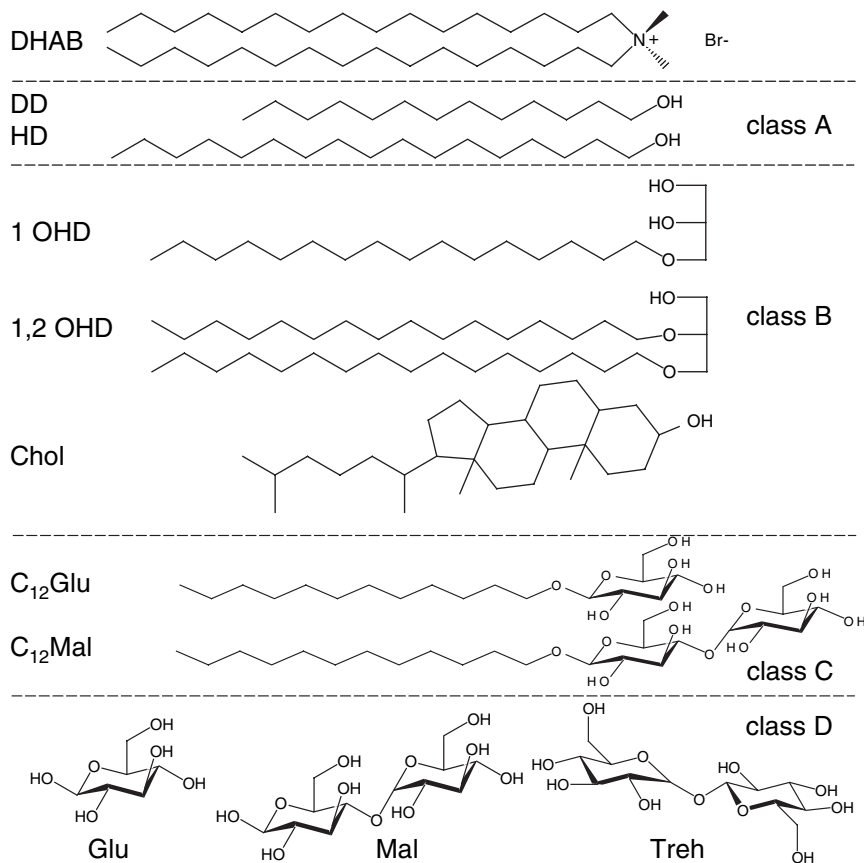
Scheme 2. Structure of DHAB (top) and the four classes of additives A–D: (A) DD is *n*-dodecanol and HD is *n*-hexadecanol; (B) 1-OHD is 1-O-*n*-hexadecyl-rac-glycerol, 1,2-OHD is 1,2-O-di-*n*-hexadecyl-rac-glycerol and Chol is cholesterol; (C) C_{12}Glu is *n*-dodecyl-β-D-glucopyranoside, and C_{12}Mal is *n*-dodecyl-β-maltoside; (D) Glu is glucose, Mal is maltose and Treh is trehalose

6-Nitrobenzisoxazole-3-carboxylate (6-NBIC) was synthesized according to a literature procedure[10,11] and was stored at $-20°C$.

Kinetic measurements

The vesicular solutions were prepared by weighing the appropriate amounts of DHAB and additive and then dissolving them in doubly distilled water. The samples were left at $50°C$ for 1 h and then sonicated at $50°C$ for 15 min (until a homogeneous, bluish solution was obtained).

The decarboxylation of 6-NBIC was monitored at $35.0 \pm 0.1°C$ (which is above the main phase transition temperature for DHAB vesicles of $28°C$[12]) by measuring the increase in the absorption at 410 nm for at least five half-lives of each of the samples. The samples contained 0.9 ml of DHAB + additive, NaOH (100-fold excess of 6-NBIC) and a stock solution of 6-NBIC (4 µl of 6 mM stock). Each of the kinetic measurements in the vesicular solutions was repeated five times.

Differential scanning microcalorimetry

All DSC measurements were carried out using a CSC NANO II differential scanning microcalorimeter as described previously.[13] A total amphiphile concentration of 2 mM and 2.25 mM sodium hydroxide were used for the experiments. Five scans were taken between 5 and $100°C$ at a scan rate of $1°C$ min^{-1}. The reference cell contained doubly distilled water. The first scan was neglected due to the thermal history of the machine, but the other scans were all identical.

Cryo-electron microscopy measurements

Aliquots of a 5 mM amphiphile solution were deposited on holey carbon grids in a controlled environmental chamber at $35°C$ and 100% humidity. The excess solution was blotted off using filter paper and the samples were vitrified by rapid plunging into liquid ethane. The micrographs were recorded on a Philips CM 120 cryo-electron microscope operating at 120 kV and using low-dose conditions.

RESULTS AND DISCUSSION

Decarboxylation of 6-NBIC

The rate constants for the unimolecular decarboxylation of 6-NBIC were measured in the presence of DHAB vesicles and analyzed using the pseudophase model developed by Menger et al.[14] According to the model the observed rate constant of a unimolecular reaction can be described by:

$$k_{obs} = \frac{k_w + k_{ves}K_{6-NBIC}[Amph]}{1 + K_{6-NBIC}[Amph]} \qquad (1)$$

in which k_{obs}, k_w and k_{ves} are the observed, aqueous and vesicular rate constants, respectively; and K_{6-NBIC} is the binding constant of the organic substrate to the vesicular surface.

In all experiments the minimum concentration of DHAB was 9 mM, which is above the CVC (critical vesicle concentration) of DHAB.[13] At these concentrations the 6-NBIC substrate is completely bound to the vesicular interface owing to the relative excess of DHAB and the high binding constant of 6-NBIC ($>10^3$ M^{-1}).[6] The strong binding originates mainly from electrostatic interactions between the substrate and the oppositely charged vesicles, but also from hydrophobic interactions. Therefore, under these conditions it can be assumed that $K_{6-NBIC}[Amph] \gg 1$ and $k_{ves}K_{6-NBIC}[Amph] \gg k_w$. Consequently, Eqn (1) can be simplified to:

$$k_{obs} = k_{ves} \qquad (2)$$

In Fig. 1 the relative rate constants (k_{ves}/k_0) as a function of the mole fraction of additive are shown for the different classes of additives. Rate constants for several different mole fractions are reported in Table 1. As a reference, the vesicular rate constant in the absence of additive was taken to be k_0. It is apparent that due to the addition of most of the additives (class A and B) the rate constant of the decarboxylation reaction decreases. Figure 1(A) shows that the addition of n-dodecanol (DD) has a larger inhibiting effect than the addition of n-hexadecanol (HD). It has been shown previously that n-decanol and n-hexadecanol in DHAB at $35°C$ lead to a similar decrease in the rate constant for the 6-NBIC decarboxylation.[12] However, at higher concentration the reaction in the presence of n-decanol was slower than that with hexadecanol. Under similar conditions the magnitudes of the vesicular rate constants for vesicles containing HD were the same (within error), however the effect of DD is more pronounced than that of n-decanol. This would suggest that DD has a somewhat larger perturbing effect on the vesicular interface. Figure 1(B) compares the class B additives. Vesicles containing the double-tailed glycerol ether 1,2-O-di-n-hexadecyl-rac-glycerol (1,2-OHD) were less stable compared to vesicles with other additives, therefore the errors are larger in this case. However, 1,2-OHD seems to decrease the rate constant more than the single-tailed glycerol ether 1-O-n-hexadecyl-rac-glycerol (1-OHD). Cholesterol (Chol) induces the largest inhibiting effect, decreasing the vesicular rate constant by a factor of 3 at 50 mol.%. Figure 1(C) compares the kinetic effects of the class C additives. It can be seen that n-dodecyl-β-maltoside (C$_{12}$Mal) does not follow the approximately linear relationship between the natural logarithm of the rate constant and mole fraction, as found for the other additives. All points for C$_{12}$Mal show a smaller decrease
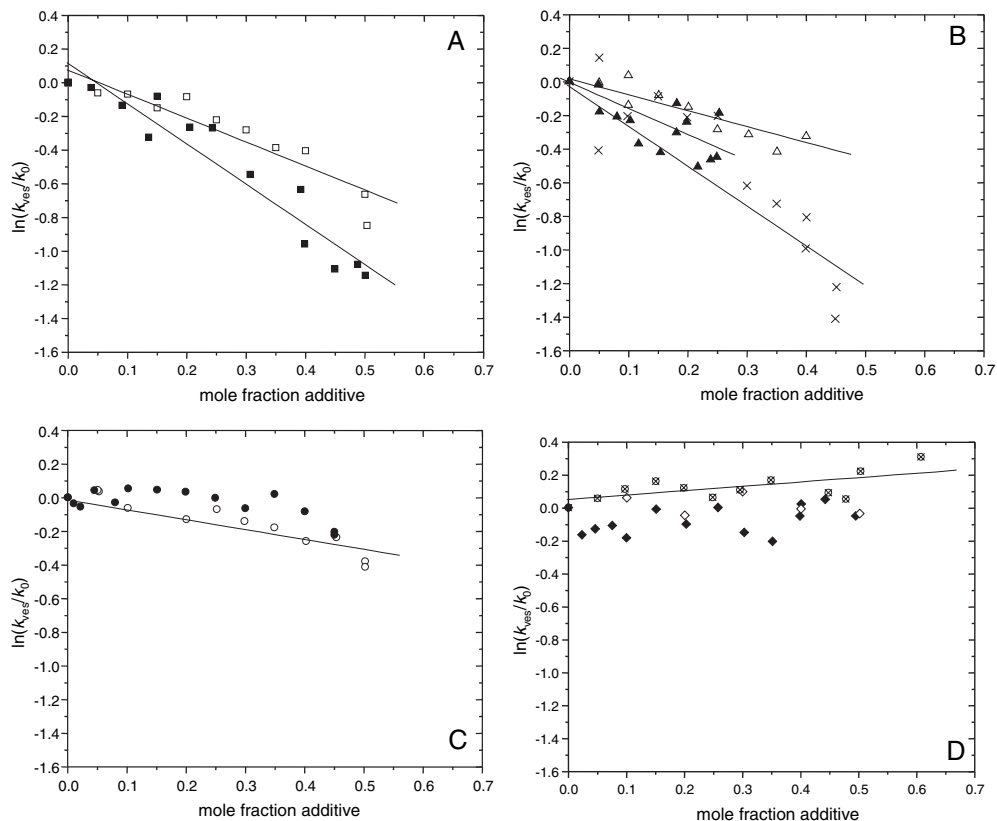
Figure 1. Plots of $\ln(k_{ves}/k_0)$ versus the mole fraction of the additives in the DHAB vesicles: (A) the additives are HD ($\square$) and DD ($\blacksquare$); (B) the additives are 1-OHD ($\triangle$), 1,2-OHD ($\blacktriangle$) and Chol (x); (C) the additives are C_{12}Glu ($\bigcirc$) and C_{12}Mal ($\bullet$); (D) the additives are Treh ($\otimes$), Glu ($\blacklozenge$), and Mal ($\diamond$). The lines are drawn to guide the eye. We note that HD, DD and Chol are above the T_m at 30 mol.%

in the rate constant in comparison with n-dodecyl-β-D-glucoside (C_{12}Glu). Figure 1(D) compares the class D additives and shows that the only unfunctionalized sugar that affects the rate constant positively is trehalose (Treh),

Table 1. Vesicular rate constants for all additives at different mole percentages (all in liquid-crystalline state); the value for pure DHAB vesicles is $8.6 \times 10^{-3}\,s^{-1}$

|	k_{ves} for different mol.% ($\times 10^{-3}\,s^{-1}$)		
Additive	10 mol.%	20 mol.%	30 mol.%
HD	8.0	7.9	6.5
DD	7.5	6.6	5.0
1-OHD	7.9	7.4	6.3
1,2-OHD	6.8	6.8	5.5[a]
Chol	7.0	7.0	4.2[b]
C_{12}Glu	8.0	7.6	7.5
C_{12}Mal	9.1	8.9	8.1
Treh	9.7	9.7	9.6
Glu	7.2	7.8	7.4
Mal	9.1	8.2	9.0

[a] Measured at 25 mol.%.
[b] Measured at 35 mol.%.

but the effects are so small that no definite conclusions can be drawn.

When the vesicular rate constants in the presence of DD, C_{12}Glu and C_{12}Mal are compared, it can be concluded that k_{ves} is highest for the sugar-based additives. This slight increase in k_{ves} relative to vesicles containing DD is probably a result of the (partial) displacement of water from the Stern region by the sugar moieties.[15] Displacement of water molecules from the Stern region has been shown for mixed micelles of sodium n-dodecylsulfate and a sugar-based surfactant.[16] Recently it was found, using reaction kinetic experiments, that this is also the case for vesicles composed of C_{12}Glu, DHAB and POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine).[17] The 6-NBIC decarboxylation reaction is sensitive towards hydrogen bonding. Hence, if there are fewer possibilities for hydrogen bonding to the organic substrate, the rate constant should increase. However, considering that the vesicular rate constants are lower compared to that for DHAB vesicles in the absence of the additives, the beneficial effect on the vesicular rate constants by the sugar groups is largely counteracted by the effect of the tails of the sugar-based additives. This is exemplified by the fact that DD has a
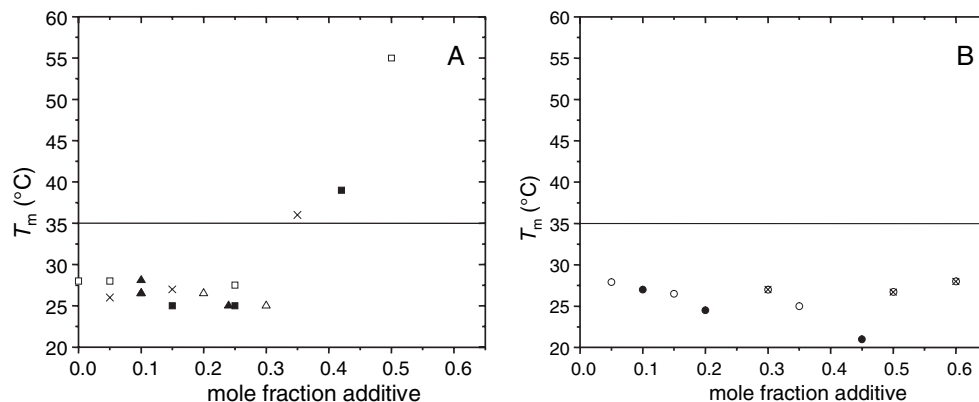
Figure 2. Main phase transition temperatures of DHAB vesicles in the presence of: (A) DD (■), HD (□), Chol (x), 1-OHD (△), 1,2-OHD (▲); (B) C_{12}Glu (○), C_{12}Mal (●) and Treh (⊗). The horizontal lines represent the temperature at which the kinetic experiments were carried out

larger effect on the vesicular rate than C_{12}Glu and C_{12}Mal. Similarly, when vesicular solutions containing HD and 1-OHD (class B) are compared, the vesicular rate constants are slightly higher in the presence of 1-OHD than in the presence of HD. (The head group of 1-OHD has the formula $C_3H_7O_3$, which can be considered half a glucose head group.)

The effects of glucose and maltose on the rate constants are not significant because these sugars do not bind to the vesicular interface. Despite the fact that trehalose is known to bind to the vesicular surface[18,19] in the concentration range used in this study, the kinetic response for this additive is very minor. However, all k_{ves} values found for Treh/DHAB solutions were faster than those for pure DHAB, which can be taken as a further indication of replacement of water molecules from the vesicular surface.[20–23]

Vesicles containing DD show a larger effect on the decarboxylation reaction than vesicles with HD. This could be due to tail matching/mismatching.[12] Tails of additives that match in length with the tails of the double-tailed amphiphiles have less impact on the packing of the vesicular bilayer than in the case of mismatching tails, therefore mismatching amphiphile tails are expected to have a stronger negative effect on the vesicular rate constant than in the case of matching tails.

An important bio-relevant additive in class B is cholesterol. At low concentrations cholesterol induces homophospholipid association in fluid bilayers, which is a direct result of the hydrophobic effect.[24] Cholesterol is said to create organization in lipid bilayers.[25–27] Upon inclusion of cholesterol in the membrane the interamphiphile interactions decrease and this increases the hydration of the interface.[28] Cholesterol has the largest hydrophobic surface area of all additives used in this study. This leads to a deeper penetration of cholesterol into the bilayer than is the case for the other additives. As a result, the packing of the vesicle is more strongly influenced by cholesterol and leads to the largest decrease in k_{ves}.

Differential scanning calorimetry measurements

In order to obtain more details about the aggregates formed from amphiphiles and the additives, differential scanning microcalorimetric experiments were performed to measure the main phase transition temperatures (T_m) as a function of the composition of the bilayers (Fig. 2). The trends in the T_m of vesicles formed from DHAB and additives used in this study are similar to those found for mixed vesicles formed from dimethyl-di-n-octadecylammonium chloride and organic additives.[29]

However, upon addition of small amounts (<30 mol.%) of all additives a slight decrease in T_m was found. Above 30 mol.%, C_{12}Mal and C_{12}Glu both give a further decrease in the main phase transition temperature upon increasing the mole fraction of additive. However, cholesterol, DD and HD show an increase in T_m at high mole fractions of additive. The higher the melting point of the pure additive, the higher T_m becomes. The majority of the additives induce phase transitions that occur below 35°C, therefore the chemical behavior of the mixed vesicles can be compared to one another at this temperature because the vesicles are all in the liquid crystalline state. Unfortunately the data do not allow a definite conclusion regarding a correlation between the effects of the additives on the catalysis (k_{ves}) of the 6-NBIC decarboxylation and on the phase transition temperature of the tails (T_m).

Activation parameters

For a further characterization of the catalytic effects induced by mixed vesicles the isobaric activation parameters were measured for a selection of vesicles containing 50 mol.% of the additives. The enthalpies and entropies of activation were calculated using:[30]

$$\ln\left(\frac{k_{ves}h}{k_B T}\right) = \frac{\Delta^{\ddagger}S^{\ominus}}{R} - \frac{\Delta^{\ddagger}H^{\ominus}}{RT} \quad (3)$$
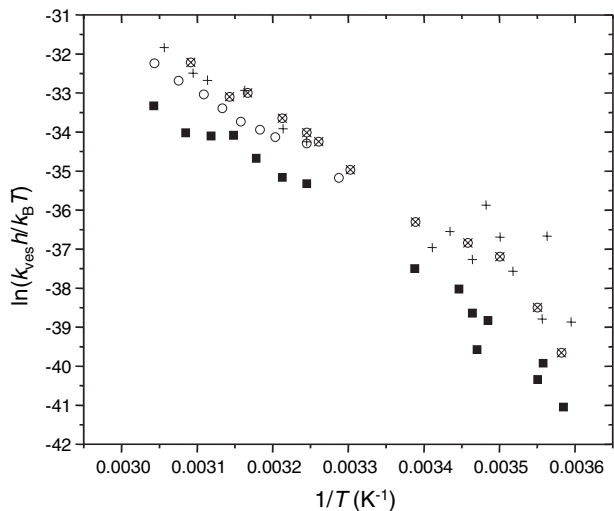
Figure 3. Eyring plot for the decarboxylation of 6-NBIC in DHAB vesicles containing 50 mol.% DD (■), 50 mol.% C_{12}Glu (○), 100 mol.% DHAB (+) and 50 mol.% Treh (⊗)

in which k_B is the Boltzmann constant, h is the Planck constant, T is the absolute temperature, R is the gas constant, $\Delta^{\ddagger}S^{\ominus}$ is the entropy of activation and $\Delta^{\ddagger}H^{\ominus}$ is the enthalpy of activation.

Eyring plots (Fig. 3) show that there is a break in the slopes around the main phase transition temperature. The data points above T_m are of good quality and therefore these slopes were used for calculations of the activation parameters. Below the T_m the turbidity of the vesicular solutions increases and the stability of the samples decreases. Hence, the quality of the raw kinetic data is less satisfactory, leading to more scattering of the data points. This is a common feature of vesicular catalysis.[5]

In Fig. 4 the calculated activation parameters at 35°C are shown, because this is the temperature at which most of the kinetic experiments were performed. In agreement with the literature, the main contribution to the Gibbs energy of activation comes from the enthalpy.[31] The errors in the calculated values for $T\Delta^{\ddagger}S^{\ominus}$ are rather large.
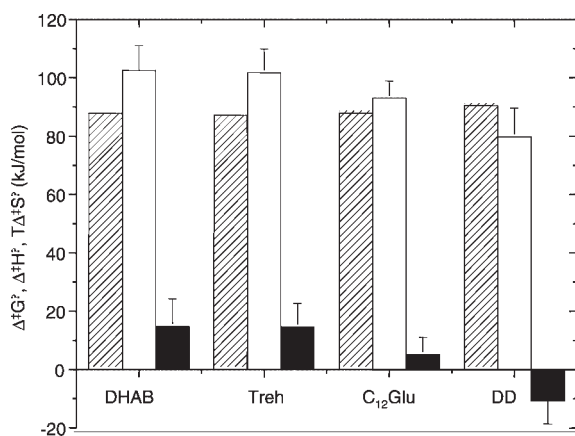
Figure 4. Activation parameters for DHAB vesicles containing 50 mol.% of different additives: $\Delta^{\ddagger}G^{\ominus}$ (lined bar), $\Delta^{\ddagger}H^{\ominus}$ (open bar) and $T\Delta^{\ddagger}S^{\ominus}$ (filled bar)

The entropy of activation only provides a minor contribution to the Gibbs energy of activation, however it does compensate in part for the enthalpy of activation. As was seen from the DSC data, vesicles containing DD have a T_m that lies above 35°C at 50 mol.%. Although the values given for $\Delta^{\ddagger}G^{\ominus}$ (and the other activation parameters) were calculated at 35°C, these data were obtained using the slope for the temperatures above T_m. The correlation between these data points is good enough to make the calculated activation parameters of the different DHAB/additive systems comparable; DD has a negative $T\Delta^{\ddagger}S^{\ominus}$ contribution, C_{12}Glu is associated with a slightly positive value for $T\Delta^{\ddagger}S^{\ominus}$ and DHAB and trehalose have approximately the same entropic contribution. Thus the vesicular solutions that have the largest k_{ves} values also have the largest values calculated for the enthalpy of activation and the largest positive $T\Delta^{\ddagger}S^{\ominus}$ contribution.

Cryo-transmission electron microscopy

Several cryo-TEM pictures were taken of mixed DHAB–C_{12}Mal vesicles because single-tailed surfactants can solubilize vesicles into mixed micelles. The cryo-TEM pictures of 5 mM total concentration of DHAB vesicles containing 0, 10 and 45 mol.% C_{12}Mal were taken at 35°C. As can be seen from the kinetic data, the maximum observed rate constant for the decarboxylation reaction is observed at ca. 10 mol.% C_{12}Mal (Fig. 1C). In the absence of C_{12}Mal (Fig. 5A) and in the presence of 10 mol.% C_{12}Mal (Fig. 5B) the vesicles are spherical. No micelles are observed. However, at 45 mol.% C_{12}Mal, holes and discontinuations in the bilayer can be seen (Fig. 5C). This will affect the overall hydration of the interface and therefore influence the decarboxylation reaction of 6-NBIC, consistent with the observed kinetic data. As long as the vesicles are still spherical, the reaction is accelerated by the presence of C_{12}Mal. When the bilayer is disturbed (structures containing more defects) the vesicular rate constant decreases. Upon a further increase of the C_{12}Mal content the bilayer is even further disturbed and hence the vesicular rate constant decreases even more.

SUMMARY

In this study a kinetic analysis of the decarboxylation of 6-NBIC in DHAB vesicles in the presence of sugars, long-tailed sugars, cholesterol and alcohol additives has been made. One of the additives to DHAB vesicles leads to an increase in the vesicular rate constant relative to DHAB vesicles, and three of the additives have no significant effect on the rate constants. All other additives studied lead to a decrease in the vesicular rate constant. The natural logarithm of the vesicular rate constants
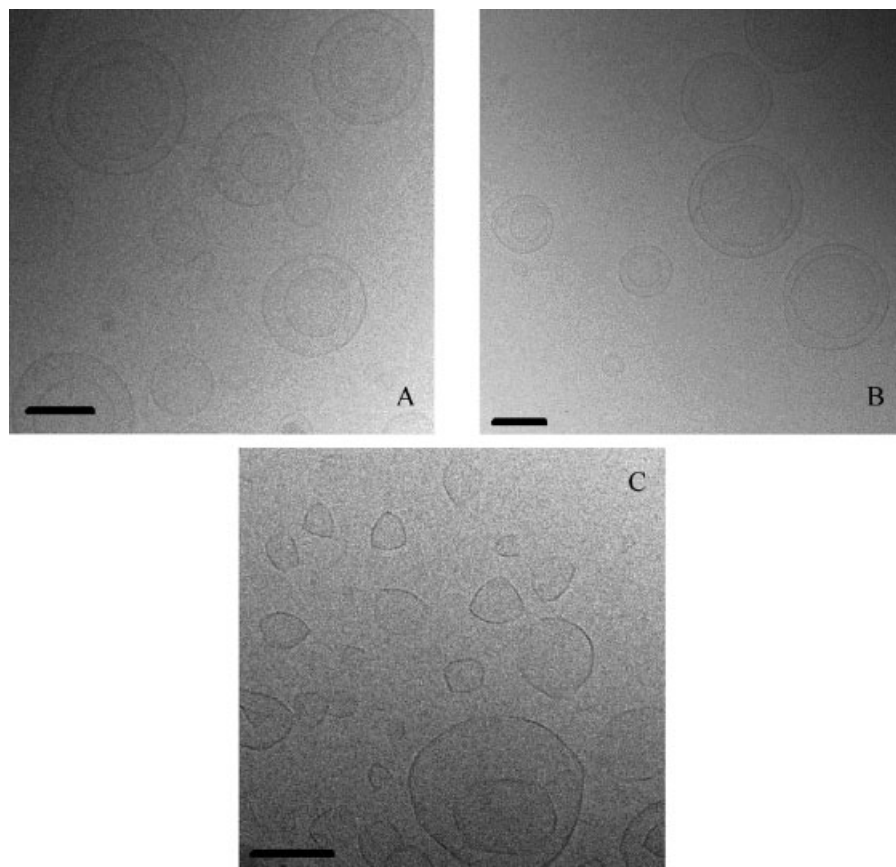
Figure 5. Cryo-TEM pictures: (A) 100 mol.% DHAB in 2.25 mM NaOH at 35°C; (B) 10 mol.% C_{12}Mal; (C) 45 mol.% C_{12}Mal. The bars represent 100 nm

measured varies linearly with the mole fraction of additive for most additives. The monohydric single-tailed alcohols and cholesterol showed the largest decrease of k_{ves}. The glycerol ethers and the sugar-based surfactants showed behavior in-between that of the monohydric single-tailed alcohols and the sugars, which seemed to have practically no effect on the rate constant. The sugar-based surfactants induced larger vesicular rate constants than the glycerol ethers.

In the case of C_{12}Mal the relationship between k_{ves} and the mole fraction of additive was not linear. Cryo-TEM showed that when (partial) solubilization does not yet play a role for mixed vesicles containing DHAB and C_{12}Mal the rate constant is increased relative to the vesicular solution containing only DHAB. Once the C_{12}Mal starts inducing defects in the vesicles, the rate constant becomes smaller than that in pure DHAB solutions.

A likely explanation for the observed increase in the vesicular rate constant of vesicles containing C_{12}Mal and C_{12}Glu compared to vesicles containing DD is that water is being displaced from the interface by the sugar hydroxyl groups. This effect is known from the literature.[15,16] The hydrophobic tails of DD, C_{12}Mal and C_{12}Glu have the ability to change the overall packing of the aggregate and possibly alter the hydration (increase

in water concentration) of the vesicular interface, thereby slowing down the vesicular rate constant. In the case of the n-alkyl pyranosides a balance between the positive effect of the water displacement and the negative effect of the membrane disruption caused by the tails seems to determine the magnitude of the vesicular rate constant. The hydroxyl groups of the glycerol ether probably also have the capability to displace water but because there are fewer hydroxyl groups at the interface the positive effect is less pronounced.

The DSC measurements indicate that most DHAB/ additive compositions have a T_m below the temperature at which the kinetic measurements were carried out, therefore the kinetic data can be compared because all systems are in the liquid-crystalline state (at <30 mol.% additive). The Eyring plots show that the enthalpy of activation is the largest contributor to $\Delta^\ddagger G^\ominus$. The additives with the highest values for k_{ves} (pure DHAB and trehalose) also had the largest positive value for $T\Delta^\ddagger S^\ominus$.

Acknowledgments

REFERENCES

1. Pross A. *Origins Life Evol. Biosphere* 2004; **34**: 307–321.
2. Eliasz AW, Chapman D, Ewing DF. *Biochim. Biophys. Acta* 1976; **448**: 220–230.
3. Kemp DS, Paul KG. *J. Am. Chem. Soc*. 1975; **97**: 7305–7312.
4. Grate JW, McGill RA, Hilvert D. *J. Am. Chem. Soc*. 1993; **115**: 8577–8584.
5. Germani R, Ponti P, Romeo T, Savelli G, Spreti N, Cerichelli G, Luchetti L, Mancini G, Bunton CA. *J. Phys. Org. Chem*. 1989; **2**: 553–558.
6. Kunitake T, Okahata Y, Ando R, Shinkai S, Hirakawa S. *J. Am. Chem. Soc*. 1980; **102**: 7877–7881.
7. Talhout R, Engberts JBFN. *Langmuir* 1997; **13**: 5001–5006.
8. Scarpa MV, Araujo PS, Scheier S, Sesso A, Oliveira AG, Chaimovich H, Cuccovia IM. *Langmuir* 2000; **16**: 993–999.
9. Balzer D. *Langmuir* 1993; **9**: 3375–3384.
10. Borsche W. *Chem. Ber*. 1909; **42**: 1316.
11. Lindemann H, Cisse H. *Ann. Chem*. 1929; **469**: 44.
12. Blandamer MJ, Briggs B, Cullis PM, Irlam KD, Kirby SD, Engberts JBFN. *J. Mol. Liq*. 1998; **75**: 187.
13. Linthorst JA, Klijn JE, Engberts JBFN. *Int. J. Mol. Sci*. 2005; **6**: 104–118.
14. Menger FM, Portnoy CE. *J. Am. Chem. Soc*. 1967; **89**: 4698–4703.
15. Bernik DL, Zubiri D, Tymczyszyn E, Disalvo EA. *Langmuir* 2001; **17**: 6438–6442.
16. Griffiths PC, Pettersson E, Stilbs P, Cheung AYF, Howe AM, Pitt AR. *Langmuir* 2001; **17**: 7178–7181.
17. Klijn JE, Engberts JBFN. *Langmuir* 2005; **21**: 9809–9817.
18. Crowe LM, Reid DS, Crowe JH. *Biophys. J*. 1996; **71**: 2087–2093.
19. Crowe JH, Crowe LM, Oliver AE, Tsvetkova N, Wolkers W, Tablin F. *Cryobiology* 2001; **43**: 89–105.
20. Lambruschini C, Relini N, Ridi A, Cordone L, Gliozzi A. *Langmuir* 2000; **16**: 5467–5470.
21. Takahashi H, Ohmae H, Hatta I. *Biophys. J*. 1997; **73**: 3030–3038.
22. Sola-Penna M, Meyer-Fernandes JR. *Arch. Biochem. Biophys*. 1998; **360**: 10–14.
23. Kaushik JK, Bhat R. *J. Biol. Chem*. 2004; **278**: 26458–26465.
24. Cao H, Regen SL. *J. Am. Chem. Soc*. 2003; **125**: 16182–16183.
25. Mitra S, Dungan SR. *Colloids Surf. B-Biointerfaces* 2000; **17**: 117–133.
26. Veatch SL, Keller SL. *Phys. Rev. Lett*. 2002; **89**: 268101.
27. Jedloszky P, Mezei M. *J. Phys. Chem. B* 2003; **107**: 5311–5321.
28. Pasenkiewicz-Gierula M, Rog T, Kitamura K. *J. Phys. Chem. B* 2000; **78**: 1376–1389.
29. Klijn JE, Engberts JBFN. *Org. Biomol. Chem*. 2004; **2**: 1789–1799.
30. Maskill H. *The Physical Basis of Organic Chemistry*. Oxford University Press: Oxford, 1990; 247–249.
31. Patel MS, Bijma K, Engberts JBFN. *Langmuir* 1994; **10**: 2491–2492.