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Cationic Reverse Micelles Create Water with Super Hydrogen-Bond-Donor Capacity for Enzymatic Catalysis: Hydrolysis of 2-Naphthyl Acetate by α-Chymotrypsin

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Abstract: Reverse micelles (RMs) are very good nanoreactors because they can create a unique microenvironment for carrying out a variety of chemical and biochemical reactions. The aim of the present work is to determine the influence of different RM interfaces on the hydrolysis of 2-naphthyl acetate (2-NA) by α-chymotrypsin (α-CT). The reaction was studied in water/benzyl-*n*-hexadecyldimethylammonium chloride (BHDC)/benzene RMs and, its efficiency compared with that observed in pure water and in sodium 1,4-bis-2-ethylhexylsulfosuccinate (AOT) RMs. Thus, the hydrolysis rates of 2-NA catalyzed by α-CT were determined by spectroscopic measurements. In addition, the method used allows the joint evaluation of the substrate partition constant K_p between the organic and the micellar pseudophase and the kinetic parameters: catalytic rate constant k_{cat}, and the Michaelis constant K_M of the enzymatic reaction. The effect of the surfactant concentration on the kinetics parameters was determined at constant $W_0 = [H_2O]/[surfactant]$, and the variation of W_0 with surfactant constant concentration was investigated. The results show that the classical Michaelis–Menten mechanism is valid for α-CT in all of the RMs systems studied and that the reaction takes place at both RM interfaces. Moreover, the catalytic efficiency values k_{cat}/K_M obtained in the RMs systems are higher than that reported in water. Furthermore, there is a remarkable increase in α-CT efficiency in the cationic RMs in comparison with the anionic system, presumably due to the unique water properties found in these confined media. The results show that in cationic RMs the hydrogen-bond donor capacity of water is enhanced due to its interaction with the cationic interface. Hence, entrapped water can be converted into "super-water" for the enzymatic reaction studied in this work.

Keywords: enzyme catalysis · hydrolysis · interfaces · micelles · surfactants

Introduction

Several biological phenomena occur at interfaces rather than in homogeneous solution. In particular, interface/protein interactions play a key role in reactions involving membrane-bound proteins. In this regard, even though reverse micelles (RMs) are an oversimplified model, the very large interfacial region provided by these systems can be expected to enhance some effects, such as hydrogen-bonding interactions between peptide bonds, because in these media the amphipathic essence of a biological membrane is preserved.[1] Among the RMs formed by anionic surfactants, the best known are those derived from sodium 1,4-bis-2-ethylhexylsulfosuccinate (AOT) in different nonpolar media. AOT has a well-known V-shaped molecular geometry and forms stable RMs without co-surfactant. The cationic surfactant benzyl-*n*-hexadecyldimethylammonium chloride (BHDC) also forms RMs in benzene without addition of a co-surfactant and has properties that are characteristic of other RMs systems. They can encapsulate water in their polar interior, water is solubilized up to $W_0 = [water]/[BHDC] \approx 25$, and the size of the RMs increases with increasing water content W_0.[2–4] Furthermore, the properties of the water become similar to those of bulk water only when the amount of water exceeds that required for surfac-

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tant solvation.[2,3] Recently,[5] we have shown that the water properties are different for water molecules sequestrated inside anionic and cationic RM systems. This is because the water molecules entrapped inside BHDC RMs media appear to be non-electron-donating and more hydrogen-bond-donating owing to their interaction with the polar head group of the cationic surfactant. On the other hand, water molecules sequestrated inside AOT RMs show enhanced electron-donor ability in comparison with bulk water. Thus, we suggest that RMs are highly suitable for use as nanoreactors, since the properties of water can depend significantly on the kind of surfactant from which they are prepared.

Many studies on enzyme kinetics in RM solutions have been reported,[6–17] and recently they were reviewed by us.[18] In most of them, α-chymotrypsin (α-CT),[19–24] a hydrophilic and globular enzyme that is totally associated with the micelles and substrates partitioned between the micelles and the external solvent, was used. In these cases, it was found that the enzymatic activity is often substantially higher than in aqueous buffer solutions, a phenomenon known as "superactivity".[18] Falcone et al. studied the kinetics of hydrolysis of 2-naphthyl acetate (2-NA) catalyzed by α-CT in RM solutions formed by glycerol (GY)–water (38% v/v) mixture/AOT/*n*-heptane by means of spectroscopic measurements.[17] They showed that addition of GY to the micelle interior results in improved catalytic properties of α-CT, because GY addition to the RM media result in a decrease in conformational mobility of α-CT, which leads to increased enzyme stability and activity.[17,20]

On the other hand, there is some controversy about the causes of enzymatic superactivity inside RMs media. It is believed that the increased conformational rigidity of the enzyme promoted by the surfactant layer and the increased concentration of the substrate at the reaction site can contribute to the RM effect. Also superactivity has been explained in terms of the peculiar state of water in the RMs, which mimics the status of intracellular water.[18,25–29]

The goal of our work is to determine the influence of different RM interfaces on the hydrolysis of 2-NA by CT in the presence of water/BHDC/benzene RMs, and to compare the efficiency of this reaction with that observed in pure water and in the previously studied AOT RM systems.[17] The results show remarkably enhanced efficiency for α-CT in the cationic RMs in comparison with the anionic system and pure water. We will show that this is mainly because the differences in the water properties at the cationic interface make them unique for stabilization of α-CT. Thus, this water has a "super" hydrogen bond donating capacity and can interact with the enzyme at the interface. The hydrogen-bond network that can be created around the enzyme makes it more stable and increases the enzyme activity, probably by decreasing the surfactant–enzyme interaction, varying its conformation and making the active site more accessible to the substrate, with a remarkable increase in enzymatic efficiency.

Experimental Section

Materials: Benzyl-*n*-hexadecyldimethylammonium chloride (BHDC) from Sigma (>99%) was recrystallized twice from ethyl acetate. The surfactant was kept under vacuum over P_2O_5 to minimize H_2O absorption. The absence of acidic impurities was carefully checked by using 1-methyl-8-oxyquinolinium betaine (QB) as indicator, because such impurities can significantly affect the pH of the dispersed aqueous phase.[2,30]

α-Chymotrypsin (α-CT), M_w 24800, from bovine pancreas (Sigma) and 2-naphthyl acetate (2-NA, Sigma) were used as received. Benzene from Merck (fluorescence spectroscopy quality) was used, and ultrapure water was obtained from a Labonco equipment model 90901-01. The pH of the bulk water solution was maintained at 8.7 by using a 20 mM phosphate buffer. In the case of RM media, it is known that the pH cannot be measured inside the polar core of the aggregate.[31] A meaningful approximation to the pH within the aqueous pseudophase of the RMs can be made by using a pure source of BHDC and having sufficient buffering capacity in the bulk solution. In this sense, the value of the pH inside the polar core is referred to the homogeneous buffer solution and it is called pH_{ext}.

Procedures and kinetics: Solutions of BHDC in benzene were prepared by weighing and dilution. The molar ratio between water and BHDC is defined as $W_0=[H_2O]/[BHDC]$. The polar solvent was added to the micellar system by using a calibrated microsyringe.

Partition constants for 2-NA between benzene and water were determined by the hand-shaking method, using UV/Vis spectroscopy to record the decrease of the absorbance of 2-NA in water after mixing with the polar solvent at 25.0±0.1 °C.

Reactions were followed spectrophotometrically by means of the increase at the maximum of the absorption band ($\lambda_{max}=327$ nm) of the product 2-naphthol (2-N) at 25.0±0.5 °C. To start a kinetic run in homogeneous media, a stock solution of 2-NA in aqueous buffer solution was added to a thermostated cell containing α-CT in the same buffer. The concentrations of 2-NA and α-CT in the reaction media were around 10^{-4} and 10^{-6} M, respectively. In the micellar media, the stoppered cell was filled with a fixed volume of BHDC in benzene with the substrate. The desire W_0 value was reached by adding buffer solution with a microsyringe. After thermostating the cell, the enzymatic reaction was initiated by addition of a volume of α-CT dissolved in the RMs to give 3 mL of micellar solution with the desired 2-NA and α-CT concentrations.

The hydrolysis of 2-NA catalyzed by α-CT, which in the RM system is totally incorporated in the micellar pseudophase, follows the Michaelis–Menten mechanism[7,8] [Eq. (1)].

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\text{-}S \xrightarrow{k_{cat}} E + P \quad (1)$$

Applying the steady-state approximation to E–S gives the rate law of Equation (2),

$$v_0 = \frac{k_{cat}[E][S]}{(K_M + [S])} \quad (2)$$

where v_0 is the initial reaction rate (in $M s^{-1}$), [E] and [S] are the analytical enzyme and substrate concentration, respectively, k_{cat} is the catalytic rate constant, and K_M the Michaelis constant defined by Equation (3).

$$K_M = (k_{-1} + k_{cat})/k_1 \quad (3)$$

Equation (2) can be rearranged into a form that is amenable to data analysis by linear regression, known as the Lineweaver–Burk equation [Eq. (4)]

$$[E]/v_0 = (1/k_{cat}) + (K_M/k_{cat})\ 1/[S] \quad (4)$$

Equation (4) directly provides k_{cat} from the reciprocal of the intercept, and the catalytic efficiency k_{cat}/K_M from the reciprocal of the slope. K_M is obtained from the slope/intercept ratio.[7,8]

The absorbance was recorded as a function of time, and v_0 was obtained from the slope of [2-N] versus t profiles. The formation of 2-N was linearly dependent on the reaction time during the first 20 min of reaction. The v_0 values were plotted according to the Lineweaver–Burk relationship [Eq. (4)].

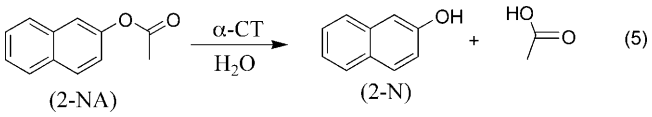
The pooled standard deviation of the kinetic data, using different samples, was less than 5%.

Results and Discussion

To the best of our knowledge the enzymatic hydrolysis of 2-NA in presence of water/BHDC/benzene RM has not been explored yet, and the reaction was only investigated in reverse micelles media made with the anionic surfactant AOT.[17]

Prior to studying the enzymatic reaction in the RM solutions, the stability of α-CT was tested by absorption and emission spectroscopy (results not shown), as was done previously for other systems.[17] The data show that the spectroscopic properties of the protein are very similar in homogeneous and micellar media, that is, encapsulation of the enzyme in the BHDC RM medium does not significantly alter the tertiary structure of α-CT, as was found previously in the AOT RM medium.[5,17,32,33]

Reactions in water: Although the enzymatic hydrolysis of 2-NA by α-CT was previously studied,[17] we tested whether under our experimental conditions the reaction is the same. UV/Vis spectroscopic analysis showed that the hydrolysis of 2-NA catalyzed by α-CT in water, produces 2-N [Eq. (5)] in quantitative yield.



(5)

The absorption spectra taken at different reaction times show a clear increase in the intensity at $\lambda = 335$ nm (not shown), evidencing formation of the product 2-N. Figure 1 shows the data treated according to a Lineweaver–Burk plot. The linearity of the plot indicates that, under the condition employed, the Michaelis–Menten mechanism applies in water at pH 8.7.[19] From the slope and the intercept of the line in Figure 1, values of the experimental kinetic parameters k_{cat}^{exp} and $(K_M^{exp})^{bulk}$ were calculated (Table 1). These values are compatible with those obtained by others[17,19] and make us confident that, under our experimental conditions, the reaction follows the same mechanism considering the different source of the employed enzyme.

Reactions in water/BHDC/benzene reverse micelles: Figure 2 shows a typical absorption spectrum for the hydrolysis of 2-NA at reaction time $t = 240$ s in BHDC RM media. The absorption spectra taken at different times of reaction show a clear isosbestic point, which evidences the lack of intermediates and/or product decomposition. As was previously found in the AOT RM system, the band at $\lambda = 335$ nm corresponds to the product 2-N.[17]

Interestingly, in BHDC RM medium a new band appears at $\lambda = 360$ nm which is absent in the AOT RMs media but it is present in pure water at pH 8.7. Thus, we attribute this band to the presence of 2-naphtholate (2-N$^-$). It seems that 2-N loses its acidic proton yielding 2-N$^-$ in BHDC RMs but

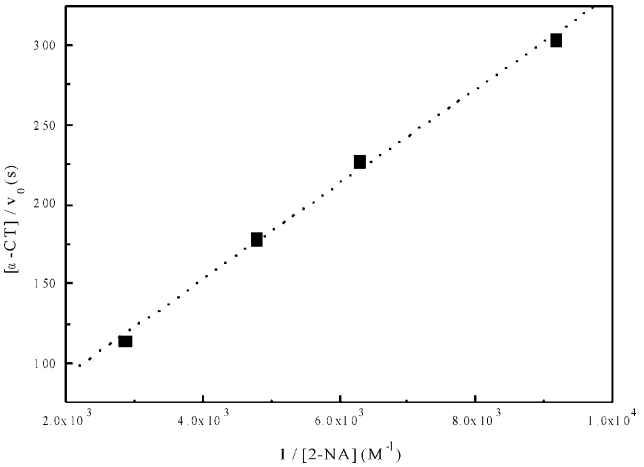


Figure 1. Lineweaver–Burk plot for the α-CT-catalyzed hydrolysis of 2-NA in water, pH 8.7 (phosphate buffer, 20 mM). [α-CT] = 2×10^{-6} M.

Table 1. Summary of experimental kinetic parameters of the enzymatic reactions in homogeneous media and in water/BHDC/benzene RMs.

Parameter	Water	Water/BHDC/benzene
k_{cat}^{exp} [s^{-1}] $\times 10^{-2}$	3.02 ± 0.13	24.13 ± 0.02
K_M^{exp} [M] $\times 10^{-4}$	9.07 ± 0.09	51.12 ± 0.23
$(k_{cat}^{exp}/K_M^{exp})$ [$M^{-1}s^{-1}$]	33	47.2
$[(K_M)_{corr}]^{bulk}$ [M]	0.06	–
$[k_{cat}^{exp}/(K_M)_{corr}]^{bulk}$ [$M^{-1}s^{-1}$]	0.5	–
$((K_M)_{corr})^{mic}$ [M]	–	4.48×10^{-3}
$[k_{cat}^{exp}/(K_M)_{corr})]^{mic}$ [$M^{-1}s^{-1}$]	–	53.81

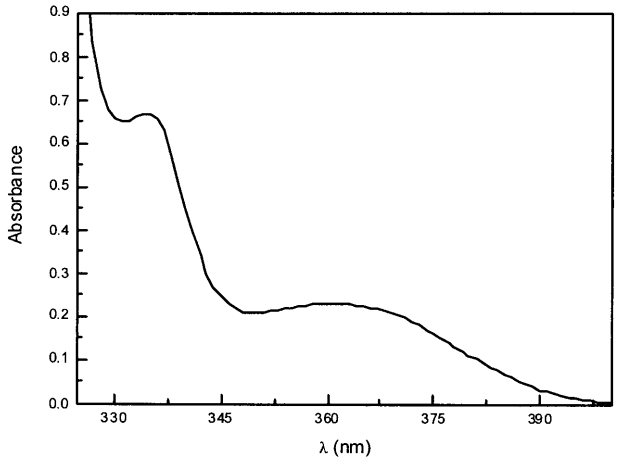


Figure 2. Representative absorbance spectrum at t = 240 s for the hydrolysis of 2-NA catalyzed by α-CT in the presence of BHDC RMs at $W_0 = 10$. [BHDC] = 0.2 M; $[\alpha\text{-CT}]_t = 2 \times 10^{-6}$ M; [2-NA] = 7.5×10^{-3} M; pH 8.7.

not in the AOT RM medium. The question is why 2-N yields 2-N$^-$ in BHDC RMs, while this process is inhibited in AOT RMs, even though the pH of the water is the same. We have demonstrated that in AOT a proton gradient exists inside the RMs toward the interface leaving the interior neutral.[31] Thus, the absence of the band that corresponds to 2-N$^-$ inside AOT RMs indicates that 2-N exists exclusively at the AOT interface where deprotonation is not favored. Quintana et al. have shown[5] that water sequestered inside anionic and cationic RM systems has different properties. Water molecules entrapped inside AOT RMs show enhanced electron-donor ability in comparison with bulk water. Thus, water at the AOT RM interface is not good for solvating anions. Furthermore, AOT is a very good hydrogen-bond acceptor,[34–37] so 2-N can undergo this interaction with the polar head of the surfactant. On the other hand, water entrapped inside the BHDC RMs appear to be non-electron-donating because of its interaction with the polar head group of the cationic surfactant. Thus, it seems that the positive charge of the surfactant and the unique interfacial water properties favor formation of 2-N$^-$ at the BHDC RM interface, and this is an interesting property of the RMs as nanoreactors. The distribution of the reaction product can be modified simply by changing the properties of the interfacial water, which can be done by choosing the right surfactant. Moreover, our results confirm the previous suggestion that the term "pH" is not suitable for the RM field, because the proton concentration depends on the RM region.[31]

Even though the product of the enzymatic reaction is not the same for BHDC and AOT RMs, deprotonation of 2-N is fast, so we still can apply Equations (2)–(4) in both micellar media.

A representative example of the plots obtained for water/BHDC/benzene according to Equation (4) is shown in Figure 3 for [BHDC] = 0.05 M and at different W_0 values. The slope and intercept of the lines are the same at all W_0 values, that is, the increased water content inside the RM does not affect the kinetic parameters. In other words, the enzymatic reaction does not occur in the water pool of the BHDC RM medium.

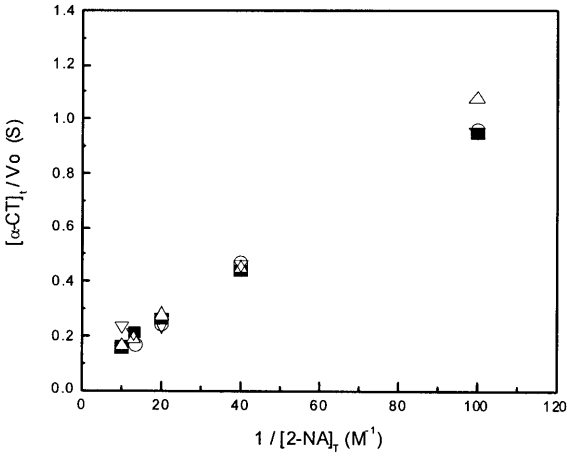


Figure 3. Lineweaver–Burk plot for α-CT-catalyzed hydrolysis of 2-NA in water/BHDC/benzene RMs at different W_0. [BHDC] = 0.05 M. W_0: ○) 5, ■;) 10, △) 15, ▽) 20.

Representative results obtained for the effect of BHDC concentration on the relationship between v_0/[α-CT] and the analytical concentration of 2-NA ([2-NA]$_T$) at $W_0 = 10$ are shown in Figure 4 for water/BHDC/benzene RMs. Interestingly, in the cationic RMs enzyme saturation with the substrate is reached at lower [2-NA]$_T$ values compared to water/AOT and water–GY/AOT RM media, and indicates a trend to lower values of $(K_M^{exp})^{mic}$ (Michaelis constant determined in terms of the analytical concentration of the substrate) for the BHDC RMs. Increasing the surfactant concentration results in a decrease in v_0/[α-CT] over the whole range of concentration studied.

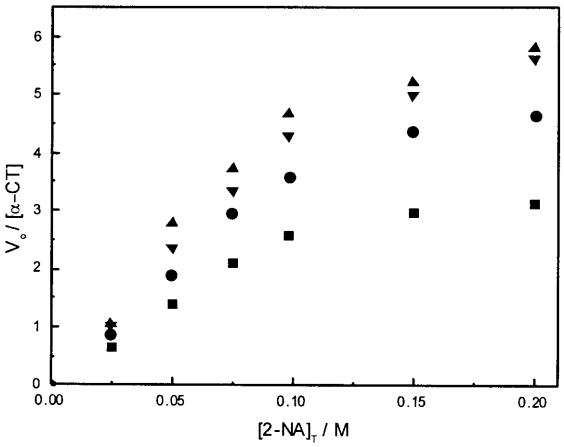


Figure 4. Effect of BHDC concentration on the relationship between the initial rate of reaction and the analytical concentration of 2-NA in water/BHDC/benzene RMs at $W_0 = 10$. [BHDC]/M: ▲) 0.10, ▼) 0.15, ●) 0.20, ■) 0.25.

The data shown in Figure 4 were plotted according to Equation (4), and the results are shown in Figure 5. The linearity of the plots at all [BHDC] considered indicates that the classical Michaelis–Menten mechanism is valid for α-CT in water/BHDC/benzene RMs, as was observed in water/AOT/*n*-heptane[17] for this and other related reactions.[6,9] From Figure 5, values of the experimental kinetic parameters in the microheterogeneous media at different surfactant concentration can be obtained, and the results are listed in Table 1 for [BHDC] = 0.2 M and $W_0 = 10$.

The dependence on BHDC concentration found in Figure 5 could have two reasons: 1) 2-NA partitioning owing to its greater solubility in benzene, which diminishes local substrate concentration in the zone in which the reaction takes place, that is, the micellar pseudophase. In other words, this is the result expected due to simple dilution, because the local concentration is inversely proportional to the surfactant concentration when the substrate is totally associated with the micellar pseudophase.[32,38] 2) Progressive inactivation of the enzyme by the surfactant diminishes the k_{cat}

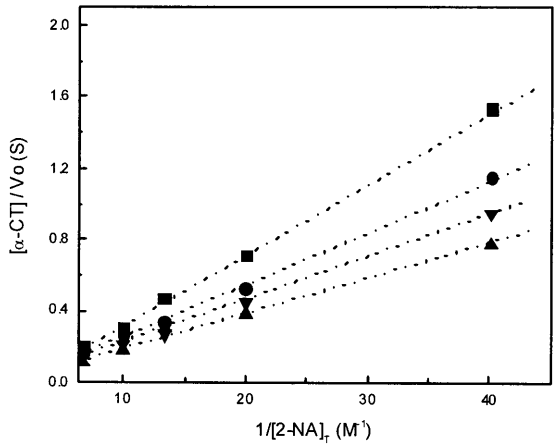


Figure 5. Effect of BHDC concentration on the Lineweaver–Burk plot for the α-CT-catalyzed hydrolysis of 2-NA in water/BHDC/benzene RMs at $W_0=10$. [BHDC]/M: ▲) 0.10, ▼) 0.15, ●) 0.20, ■) 0.25. [α-CT]$=2\times10^{-6}$ M. pH_{ext} 8.7.

value as [BHDC] increases. Figure 5 shows that all the plots have a unique intercept independent of the surfactant concentration. This fact argues against possibility 2), as expected for an enzyme totally incorporated in the RMs. Therefore, the difference in the kinetic parameters obtained from Figure 5 is due to partitioning of the substrate between the micellar pseudophase and the external solvent (K_p).

The partitioning of 2-NA between the micelles and the organic solvent pseudophases, can be treated within the framework of the pseudophases model. According to the two-pseudophase model, which considers the presence of two phases[7,39–42] (the surrounding nonpolar solvent and the microaggregates) partitioning of 2-NA between the micelles and the external solvent pseudophase, defined in Equation (6), can be expressed in terms of K_p [Eq. (7)],

$$2\text{-NA}_f + \text{BHDC} \rightleftharpoons 2\text{-NA}_b \quad (6)$$

$$K_p = \frac{[2-\text{NA}_b]}{[2-\text{NA}_f][\text{BHDC}]} \quad (7)$$

where $[2\text{-NA}]_b$ is the concentration of the substrate incorporated in the micelles, $[2\text{-NA}]_f$ the concentration of the substrate in benzene, and [BHDC] the concentration of the surfactant. This equation applies at a fixed value of W_0 and when $[2\text{-NA}]_T \ll [\text{BHDC}]$.

As was previously described,[15] it is possible to determine the K_p value from the kinetic data by using Equation (8),

$$[2-\text{NA}]_T = \frac{n}{K_p} + n[\text{BHDC}] \quad (8)$$

where $[2\text{-NA}]_T$ is the total (analytical) substrate concentration, and n the average number of substrate molecules incorporated into RMs per BHDC molecule, $n=[2\text{-NA}]_b/[\text{BHDC}]$. The proposed method is based on the assumption that, at a given W_0 value ($W_0=10$) and [enzyme]$=2\times10^{-6}$ M, the value of v_0/[enzyme] is determined only by the concentration of the substrate in the organic solvent (Figure 5). Since equal $[2\text{-NA}]_f$ implies equal n, a simple mass balance leads to the above Equation (8).[9] For a set of $[2\text{-NA}]_T$ and [BHDC] values corresponding to the same v_0/[enzyme] value, a plot of the left-hand side of Equation (8) against [BHDC] allows evaluation of n from the slope, and K_p from the slope/intercept ratio. The plot is shown in Figure 6, and the result obtained was $K_p=0.75\pm0.2\,\text{M}^{-1}$ for the water/BHDC/benzene system. This value is lower than the corresponding value obtained for water/AOT/*n*-heptane RMs[17] and thus shows greater solubility of 2-NA in benzene in comparison to *n*-heptane.

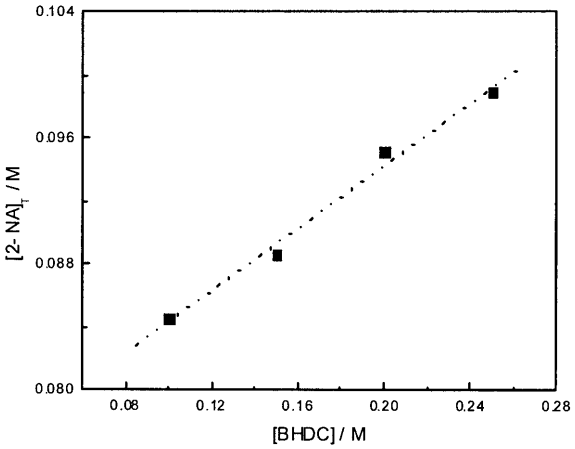


Figure 6. Experimental data from Figure 4 plotted according to Equation (8).

We also used emission spectroscopy as independent technique to obtain the K_p value in the BHDC RMs medium for comparison with the value obtained through the kinetic procedure. Thus, the partition constant K_p is quantified from the changes in the 2-NA emission spectra (not shown) with surfactant concentration.[9,39–42] The distribution of 2-NA between the micelles and the external solvent pseudophases shown in Equation (7) can also be determined from the change in the fluorescence intensity of 2-NA with surfactant concentration measured at a given wavelength.[9,39–42] Thus, the fluorescence intensity observed is given by Equation (9).

$$I = I_f + I_b \quad (9)$$

If the analytical concentration of 2-NA is kept constant and the absorbance of the sample at the working excitation wavelength is low, Equation (10) can be deduced,

$$I = \frac{I_0(\phi_f + \phi_b K_p[\text{BHDC}])}{(1 + K_p[\text{BHDC}])} \quad (10)$$

where I_0 is the incident light intensity, I_f and I_b are the fluorescent intensities when 2-NA is present in the external solvent and the disperse pseudophase, respectively, I is the fluorescence intensity measured at the surfactant concentration considered, and ϕ_f and ϕ_b are the fluorescent quantum yield of 2-NA in the organic solvent and bound to the RM interface, respectively. A K_p value of about $0.67 \pm 0.1\,M^{-1}$ is obtained from a least-squares fit of Equation (10) at $\lambda_{em} = 333$ nm (result not shown), which matches the value obtained kinetically [Eq. (8)].

The value of k_{cat}^{exp}, listed in Table 1, is practically constant at all the surfactant concentrations used at constant W_0; thus, we can assume that the shape and sizes of the RMs are not modified either by the surfactant concentration or by enzyme incorporation inside the RMs media.[17] We determined the size of the BHDC RMs using dynamic light scattering and obtained a value of 11 nm (at $W_0 = 10$) with and without enzyme, which corroborates our assumption.

The experimental kinetic parameters shown in Table 1 were corrected using Equation (11)[9,17] by partitioning of 2-NA with $K_p = 0.75\,M^{-1}$ obtained before, and the results are summarized in Table 1.

$$[(K_M)_{corr}]^{mic} = \frac{(K_M^{exp})^{mic}}{(1 + K_p[BHDC])} \quad (11)$$

The $k_{cat}^{exp}/[(K_M)_{corr}]^{mic}$ ratio obtained for water/AOT/*n*-heptane RMs is 1.14.[17] Thus, the ratio obtained in BHDC RMs (Table 1) is 47 times greater than that obtained in AOT RMs. This result is difficult to explain and it cannot be attributed solely to enzyme confinement inside RMs, because in both systems the enzyme is in a restricted environment. We think that other factors must be taken into account to explain the results. Probably, the main reason for this enzyme superactivity is the differences in the water structures at the different RMs interfaces.

Superactivity has been frequently explained in terms of the peculiar state of water in RM media, which mimics the status of intracellular water, especially water adjacent to biological membranes.[18,25–29,43] As we have previously discussed, in both RM media the reaction takes place at the interface and not in the water pool. We demonstrated recently that the water molecules in BHDC RMs are less electron donating but more strongly hydrogen bond donating than the water molecules at the AOT RM interface.[5,44] Thus, in the cationic RMs water can easily form a net of hydrogen bonds around the enzyme which prevents the enzyme–BHDC interaction, one of the most common causes of enzyme deactivation in ionic RM media.[18] Also, the hydrogen-bonding interaction can decrease the conformational mobility of α-CT, which leads to an increase in enzyme stability and activity. This has been demonstrated with polyols in homogeneous and in RM media.[15,17,18,20] It is very likely that the interaction with such super hydrogen bond donor water molecules can change the protein conformation and thus make the active site more accessible to the substrate, with the consequence that enzymatic efficiency increases.[18,45]

To make a significant comparison of the kinetic parameters obtained in BHDC RMs with the values obtained in pure water in terms of a common thermodynamic substrate-activity scale and not in terms of substrate concentrations, some correction must be applied.[17] The simplest approach is to compare the rate constants by taking as reference the external solvent of the RMs, that is, benzene. In other words, the catalytic efficiency of the enzymatic reaction obtained in the bulk solvents $(k_{cat}^{exp}/K_M^{exp})^{bulk}$ must be corrected for partitioning of the substrate between the organic and bulk polar solvents $K^{bulk}_{polar\ solvent/Bz}$ [Eq. (12)].[8]

$$[(K_M)_{corr}]^{bulk\ solvent} = (K_M^{exp})^{bulk\ solvent}/K_p^{water/benzene} \quad (12)$$

A $K_p^{water/benzene}$ value of 0.015 was obtained, and, the values of $(k_{cat}^{exp}/[(K_M)_{corr}]^{bulk}$ obtained for water are gathered in Table 1. Taking into account this correction it can be seen that the catalytic efficiency in the micellar media is almost 100 times higher than in the corresponding homogeneous media. Again, this can be attributed to the highly structured polar solvents in the inner core of BHDC RMs, which make the interfacial water a much better hydrogen-bond donor than bulk water.

In summary, the RMs seems to be very good nanoreactors because they create a unique microenvironment for carrying out a variety of chemical and biochemical reactions. In particular, the water at cationic RM interfaces seems to have enhanced hydrogen-bond donor capability, such that is transformed into "super-water" for the enzymatic reaction studied in this work.

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