The Effect of Increasing the Hydrophobicity of Penicillin on its Micelle-catalysed Hydrolysis

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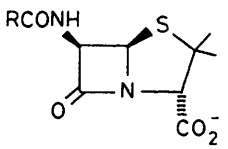
Micelles of cetyltrimethylammonium bromide catalyse the alkaline hydrolysis of alkylpenicillins and benzylpenicillin methyl ester. The equilibrium constant for binding the alkylpenicillin to the micelle and the rate constants have been obtained. Binding increases with increasing alkyl chain length but shows a non-linear dependence upon the Hansch π-value and the rate reaches a maximum value with heptylpenicillin. The free energy of transfer of a methylene group from water to the micelle shows a maximum value of 0.71 kcal mol^{-1}. The negative charge of the carboxylate group of penicillin is not necessary for catalysis.

THE attraction of organic molecules into a micelle can be due to both electrostatic and hydrophobic interactions.[1] It has been demonstrated that the rate of the hydroxide-ion catalysed hydrolysis of benzylpenicillin (I; R = $PhCH_2$) in the presence of micelles of cetyltrimethylammonium bromide (CTABr) is sensitive to electrolytes,[2] supporting the idea of electrostatic interactions between substrates and the micelle surface. In an attempt to demonstrate non-electrostatic effects the 6β-side chain of penicillin (I) has been modified in such a way as to increase the substrate lipophilicity and hence the micelle–substrate hydrophobic interaction.



(I)

Variations in substrate structure have been shown to greatly influence micellar catalysis. For example, sodium dodecyl sulphate catalyses the hydrolysis of methyl orthobenzoate but not that of methyl orthoformate.[3] The hydrolysis of *p*-nitrophenyl esters in the presence of bile salts exhibits an increase in the binding constant with increasing chain length but the rate of the hydroxide-ion catalysed hydrolysis is retarded relative to the rate in aqueous solution.[4] It was suggested that the slow rate in the presence of bile salts was due to the ester being incorporated into the micelle interior where hydroxide ion was excluded. Other examples where increasing chain length of the substrate increases micellar effects include the basic hydrolysis of *p*-nitrophenyl esters [5] and the hydrolysis of substituted phenyl carboxylates by imidazole containing cationic surfactants.[6] In fact, it appears to be quite general that increasing the lipophilicity of a substrate increases the micellar effect for both catalysis and inhibition.

EXPERIMENTAL

Benzylpenicillin methyl ester and penicillanic acid were prepared as previously described.[7]

Potassium 2-Ethylhexanoate.—Potassium (6.12 g) was dissolved in n-butanol (20 cm^3), 2-ethylhexanoic acid (25 cm^3) added, and the solution allowed to stand for 30 min.† n-Butanol was then added to make the total volume *ca.* 50 cm^3.

6-Methylpenicillin.—6-Aminopenicillanic acid (1 g) was dissolved in 3% sodium hydrogencarbonate solution (25 cm^3) and acetone (10 cm^3). The solution was then cooled to *ca.* 0 °C ‡ and acetic anhydride (0.7 g) in acetone (5 cm^3) was added while stirring. After 1.5 h the solution was cooled and washed with ether. The aqueous layer was separated and covered with ether and acidified to pH 1.5 using 1M-HCl. The layers were separated and the aqueous layer extracted twice with ether (50 cm^3). The ether layers were combined, washed with a little saturated sodium chloride solution, and dried (Na_2SO_4). After filtering, the ether solution was concentrated to *ca.* 70 cm^3 and cooled, followed by the slow addition of 1.1 equiv. of a 50% w/v solution of potassium 2-ethylhexanoate in butanol. An oil separated out; after decanting off the ether and washing with anhydrous ether (20 cm^3) and cooling, a solid precipitate could be obtained by stirring the oil, and was recrystallised (15%) from butanol–water; m.p. 174—176 °C; $\nu_{max.}$ (Nujol) 1 780, 1 660, and 1 600 cm^{-1}; δ(D_2O) 1.51 (3 H, s, CH_3), 1.63 (3 H, s, CH_3), and 2.4 (3 H, s, CH_3CO) (Found: C, 40.4; H, 4.5; N, 9.3; S, 10.6. Calc. for $C_{10}H_{13}KN_2O_4S$: C, 40.55; H, 4.4; N, 9.45; S, 10.85%).

Other 6-Alkylpenicillins.—A solution of n-acyl chloride (0.005 mol) in acetone was added dropwise with stirring to a solution of 6-aminopenicillanic acid (0.004 mol) in 3% sodium hydrogencarbonate (25 cm^3) and acetone (10 cm^3) at a temperature below 2 °C. The solution was stirred for 1 h in an ice-bath and then for a further 2 h as the temperature warmed to ambient. The mixture was shaken with ether (100 cm^3) and the ether layer discarded.

The aqueous phase was then cooled for 15 min in an ice–water-bath, covered with ether, and stirred while the aqueous pH was reduced to 1.5 using 1M-perchloric acid. The aqueous layer was separated and extracted with ether (2 × 50 cm^3). The ether extracts were combined, washed with ice-cooled saturated brine, separated, and dried. The volume was reduced to 50 cm^3, the solution cooled in ice, and 1.0 equiv. of 50% potassium 2-ethylhexanoate in butanol was added dropwise with stirring. The work-up procedure was then as for the methyl derivative.

6-Ethylpenicillin had m.p. 178—180 °C; $\nu_{max.}$ (Nujol) 1 780, 1 680, and 1 610 cm^{-1}; δ(D_2O) 1.09 (3 H, t, side chain, CH_3), 1.51 (3 H, s, CH_3), 1.63 (3 H, s, CH_3), 2.34

† 1 h = 60 min = 3 600 s.
‡ °C = K − 273.15.

(2 H, q, CH_2CO), and 4.23 (1 H, s, 3-H) (Found: C, 40.3; H, 4.95; N, 8.2. Calc. for $C_{11}H_{15}KN_2O_4S,H_2O$: C, 40.3; H, 5.2; N, 8.55%).

6-n-Propylpenicillin had m.p. 178—181 °C; $\nu_{max.}$ (Nujol) 1 784, 1 650, and 1 590 cm^{-1}; $\delta(D_2O)$ 1.04 (5 H, m, CH_3-CH_2), 1.52 (3 H, s, CH_3), 1.60 (3 H, s, CH_3), 2.35 (2 H, t, CH_2CO), and 4.23 (1 H, s, 3-H) (Found: C, 42.05; H, 5.6; H, 8.15. Calc. for $C_{12}H_{17}KN_2O_4S,H_2O$: C, 42.05; H, 5.55; H, 8.1%).

6-n-Butylpenicillin had m.p. 175—178 °C; $\nu_{max.}$ (Nujol) 1 784, 1 660, and 1 600 cm^{-1}; $\delta(D_2O)$ 0.8—2.0 (7 H, complex, aliphatic H), 1.5 (3 H, s, CH_3), 1.52 (3 H, s, CH_3), 2.35 (2 H, t, CH_2CO), 4.21 (1 H, s, 3-H), and 5.48 (2 H, 5- and 6-H) (Found: C, 45.85; H, 5.4; N, 8.1; S, 9.4. Calc. for $C_{13}H_{19}KN_2O_4S$: C, 46.1; H, 5.6; N, 8.3; S, 9.5%).

6-Pentylpenicillin had m.p. 176—178 °C; $\nu_{max.}$ (Nujol) 1 790, 1 675, and 1 608 cm^{-1}; $\delta(D_2O)$ 0.7—2.4 (9 H, aliphatic H side chain), 1.50 (3 H, s, CH_3), 1.62 (3 H, s, CH_3), 4.24 (1 H, s, 3-H), and 5.50 (2 H, 5- and 6-H) (Found: C, 45.25; H, 6.3; N, 7.55. Calc. for $C_{14}H_{21}KN_2O_4S,H_2O$: C, 45.25; H, 6.2; N, 7.55%).

6-Heptylpenicillin had m.p. 182—184 °C; $\nu_{max.}$ (Nujol) 1 790, 1 675, and 1 605 cm^{-1}; $\delta(D_2O)$ 0.7—2.0 (10 H, aliphatic H), 1.50 (3 H, s, CH_3), 1.61 (3 H, s, CH_3), 2.28 (2 H, t, CH_2CO), 4.20 (1 H, s, 3-H), and 5.50 (2 H, 5- and 6-H) (Found: C, 49.2; H, 6.6; N, 6.9. Calc. for $C_{16}H_{25}KN_2O_4S,\frac{1}{2}H_2O$: C, 49.2; H, 6.5; N, 7.15%).

6-Nonylpenicillin had m.p. 188—190 °C; $\nu_{max.}$ (Nujol) 1 780, 1 670, and 1 600 cm^{-1}; $\delta(D_2O)$ 0.88 (3 H, CH_3CH_2), 1.30 (14 H, aliphatic H), 1.52 (3 H, s, CH_3), 1.61 (3 H, s, CH_3), 2.30 (2 H, CH_2CO), 4.23 (1 H, s, 3-H), and 5.60 (2 H, 5- and 6-H) (Found: C, 50.3; H, 7.35; N, 6.4. Calc. for $C_{18}H_{29}KN_2O_4S,H_2O$: C, 50.7; H, 7.3; N, 6.6%).

6-Undecylpenicillin had m.p. 190—193 °C; $\nu_{max.}$ (Nujol) 1 795, 1 675, and 1 605 cm^{-1}; $\delta(D_2O)$ 0.88 (3 H, t, CH_3CH_2), 1.30 (18 H, aliphatic H), 1.54 (3 H, s, CH_3), 1.62 (3 H, s, CH_3), 2.30 (2 H, t, CH_2CO), 4.26 (1 H, s, 3-H), and 5.61 (2 H, d, 5- and 6-H) (Found: C, 52.8; H, 7.6; N, 6.1. Calc. for $C_{20}H_{33}KN_2O_4S,H_2O$: C, 52.8; H, 7.7; N, 6.15%).

Kinetic Measurements.—These were carried out as previously described.[2] Fast reactions were followed using a Nortech SF-3A stopped-flow spectrophotometer. Optical density changes were determined at 262.5 nm. The signal from the photomultiplier was fed into a Datalab DL901 transient recorder which was automatically triggered and simultaneously triggered the display on a Gould Advance OS-Z50B oscilloscope. The change in absorbance with time was output from the transient recorder to a chart recorder. Pseudo-first-order rate constants were calculated as described previously.[2]

RESULTS AND DISCUSSION

The second-order rate constants for the hydroxide-ion catalysed hydrolysis of 6-substituted penicillins are given in the Table, and, within experimental error, are independent of the alkyl substituent. Conversion of the 6-amino-substituent into an acylamino-group increases the rate constant 2.5-fold and 3.5-fold at ionic strength 0.5 and 0.05, respectively. Figure 1 shows the rate–surfactant concentration profile for the base-catalysed hydrolysis of three penicillins in the presence of cetyltrimethylammonium bromide (CTABr). The rate maximum moves to a lower surfactant concentration with increasing substrate lipophilicity and the rate ' maximum ' is not constant. The observed pseudo-first-order rate constants for all 6-substituted compounds examined are given in Supplementary Publication No. SUP 23210 (12 pp.).* Increasing the 6β-acylamino chain length

Summary of the data for the hydroxide-ion catalysed hydrolysis of penicillin derivatives in the presence and absence of micelles of cetyltrimethylammonium bromide at 30 °C

Derivative	k_w^{OH} / l mol^{-1} s^{-1} [a]	k_w^{OH} / l mol^{-1} s^{-1} [b]	k_m^{OH} / l mol^{-1} s^{-1} [c]	K_s / l mol^{-1} [d]	$k_m^{OH}K_s$ / l^2 mol^{-2} s^{-1}	$k_m^{OH}/k_w^{OH(b)}$	$k_m^{OH}K_s/k_w^{OH(b)}$ / l mol^{-1}
6β-Aminopenicillanic acid	0.067	0.039	0.11	10	1.1	2.8	28
6-Methylpenicillin	0.170	0.138	0.72	40	28.8	5.2	208
6-Ethylpenicillin	0.148	0.116	1.05	90	94.5	9.1	819
6-Propylpenicillin	0.150	0.109	1.42	180	256	13.0	2 340
6-Butylpenicillin	0.157	0.116	1.68	250	420	14.5	3 625
6-Pentylpenicillin	0.147	0.121	1.92	280	538	15.9	4 452
6-Heptylpenicillin	0.151	0.126	2.55	320	816	20.2	6 464
6-Nonylpenicillin	0.140	0.132	2.32	330	766	17.6	5 808
6-Undecylpenicillin	0.134	0.115	2.06	350	721	17.9	6 265
6-Benzylpenicillin	0.157	0.137	2.20	300	660	16.1	4 830
6-Benzylpenicillin methyl ester	2.30	3.69	48.0	145	6 660	13.0	1 885

[a,b] Second-order rate constant for hydrolysis in the absence of micelles, ionic strength 0.5M (KCl) and 0.05M, respectively. [c] Apparent second-order rate constant for hydrolysis in the presence of CTABr micelles, 0.05M-NaOH and 2×10^{-4}M-penicillin. [d] Apparent binding constant of penicillin derivative to micelle.

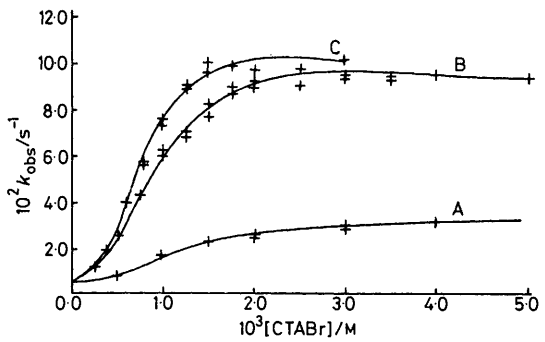


FIGURE 1 The observed pseudo-first-order rate constant for the hydrolysis of alkylpenicillins (2×10^{-4}M), with 0.05M-sodium hydroxide at 30 °C, as a function of the concentration of cetyltrimethylammonium bromide (CTABr): A, methylpenicillin; B, pentylpenicillin; C, undecylpenicillin. The lines are theoretical using the parameters given in the Table (see text)

* For details of Supplementary Publications see Notices to Authors No. 7 in *J. Chem. Soc., Perkin Trans. 2*, 1981, Index Issue.

increases the lipophilic character of the substrate and increases the binding constant, K_s, and the rate enhancement. The binding constants, K_s, were obtained using the Romsted equation (1) [8,*] with K_s as a disposable parameter to give the best fit to the experimental data. The exchange constant, K_i, for bromide ion was taken to be 25 l mol^{-1}. Values of K_s and k_m, the rate constant within the micellar phase, are given in the Table. Increasing the alkylpenicillin concentration at constant hydroxide ion and surfactant concentration resulted in a reduction in the observed pseudo-first-order rate constant, similar to the observations with benzylpenicillin.[2]

$$k_{obs.} = \frac{k_m \beta S K_s (C_D - \text{c.m.c.})}{[K_s(C_D - \text{c.m.c.}) + 1][I_t + X_t K_i]} + \frac{k_w}{K_s(C_D - \text{c.m.c.}) + 1} \quad (1)$$

Increasing the 6-acylamino chain length of the penicillin substrate not only decreases the surfactant concentration at which the maximum rate is observed but also results in a small increased maximal rate. The first effect may be rationalised on the basis of increasing affinity of the substrate for the micelle phase brought about by the increased hydrophobic interaction when the 6-acylamino side chain is increased in length. The second aspect, that of different rate maxima, must be more subtle. Increasing the surfactant concentration should eventually lead to all the substrate being associated with the micelle and, as the substrates hydrolyse in water with similar second-order rate constants, then one would expect that the same rate maximum should be obtained for each substrate, if, as generally accepted, the rate constant within the micelle is the same as that in the aqueous phase. For compounds with lower affinities for the micelle it is necessary to use higher concentrations of surfactant to incorporate all the penicillin substrate. Increasing the surfactant concentration also increases the concentration of unreactive counterion, and it is probably the displacement of reactants from the micelle surface by bromide ion that causes different rate maxima for different substrates. There is a marked increase in the pseudo-first-order rate constant below the c.m.c. of the surfactant. This is not a new phenomenon and has been noted in other systems.[9-12] Catalysis below the c.m.c. of CTABr is most predominant for the more lipophilic substrates, suggesting that induced micelle formation may be occurring.

The CTABr-catalysed hydrolysis of penicillin derivatives appears to exhibit some degree of specificity. Increasing the hydrophobicity of the 6β-side chain increases micellar catalysis (Table). The association of the penicillin substrate with the micelle is presumably the result of interactions similar to those that give micelles stability relative to their monomeric form in aqueous solution. Hence the not unexpected increase in substrate binding with increased lipophilicity of the molecule. It appears that once the 6β-side has been extended to $CH_3(CH_2)_4CONH$, further extension does not significantly increase the binding constant. It is interesting to note that there is no evidence of the longer chain compounds pulling the whole penicillin molecule into the interior of the micelle. The polar

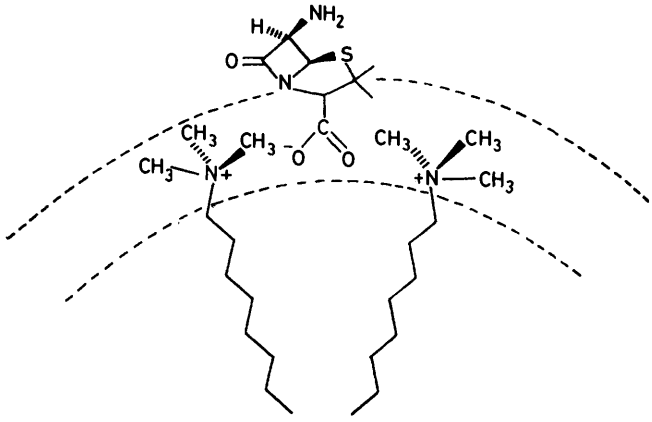


FIGURE 2 Hypothetical orientation of 6β-aminopenicillanic acid bound to micelles of CTABr

compound 6β-aminopenicillanic acid is only weakly bound to the micelle and electrostatic interactions may be all that is occurring between the substrate and micelle. Schematically, this is represented in Figure 2.

Figure 3 illustrates how increasing the length of the 6β-acylamido side chain increases the hydrophobic interaction between substrate and micelle and may thus alter the major orientation. The β-lactam carbon is

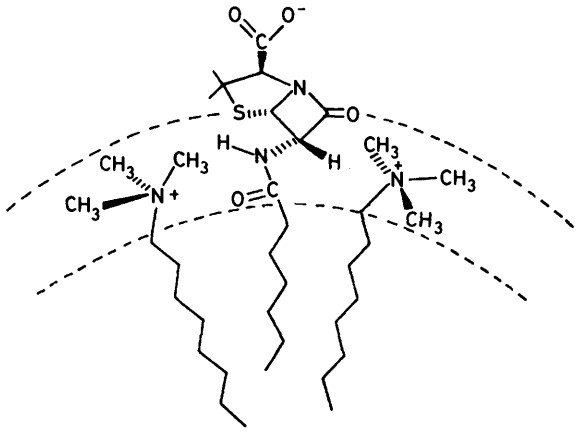


FIGURE 3 Hypothetical orientation of alkylpenicillins bound to micelles of CTABr

nicely positioned for reaction with hydroxide ion and the carbonyl oxygen of the 6-amide linkage is positioned to allow some electrostatic interaction between the electron density on the carbonyl oxygen and the micelle surface. Figure 3 also explains why forming the methyl ester of benzylpenicillin only leads to a small reduction in the micelle–substrate binding constant relative to that for

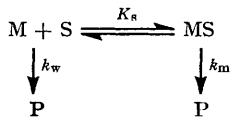
* See ref. 2 for the meaning of the symbols in equation (1).

benzylpenicillin. Because the carboxylate anion points away from the micelle surface, unlike that shown in Figure 2, it can only weakly interact electrostatically with the micelle surface.

There have been relatively few studies of the micellar-catalysed hydrolysis of amides.[13-16] The effects are small and the direction of the effect depends upon the substituent in the alkaline hydrolysis of substituted acetanilides.[13] A rate enhancement of 6 has been reported for the CTABr-catalysed hydrolysis of 4-nitroacetanilide.[16]

There is some confusion in the literature about the exact comparison that should be made when interpreting micelle and non-micelle catalysed reactions.

The commonly proposed mechanism of micellar catalysis is outlined in the Scheme where M is the micelle,



SCHEME

S the substrate, MS the micelle–substrate complex, P the product, K_s the equilibrium constant for binding, and k_w and k_m the rate constants for product formation in the bulk aqueous and micellar phases, respectively. When a comparison is made sometimes it is necessary to specify the standard states chosen or the working concentrations and sometimes it is not. If the rate constants k_w and k_m have the same units then a comparison of these numbers is independent of the standard state. The ratio k_m/k_w gives the free energy difference between the free energy of activation in the micellar phase and in the bulk aqueous phase. An apparent rate enhancement of 10^3—10^4 for bimolecular reactions can result from the higher concentration of reactants in the smaller volume of micelles and is given by $RT \ln (V_m/V_w)$, where V_m and V_w are the respective volumes of micelle and aqueous phase. This can occur even if the true rate constants within the two phases are identical. To observe this maximum rate enhancement resulting from a simple concentration effect the standard free energy of transfer of the reactant from the aqueous to the micellar phase must be more than enough to offset the loss of entropy from its restriction to a smaller volume within the micelle.

A comparison of the constant k_mK_s with k_w is dependent upon the choice of standard state because the micelle-catalysed reaction is a higher-order process. The ratio k_mK_s/k_w has units of concentration and represents the free energy of transfer of the transition state from the aqueous phase to the micellar phase.

If the substrate is modified by, say, the addition of a hydrophobic substituent then, if like is compared with like, a comparison of the rate constants is independent of the choice of standard states. Relative values of k_m give the relative free energies of binding substituents to the micelle in the ground state and transition state; relative values of K_s give the free energies of transfer of substituents from the aqueous to the micellar phase in the ground state and relative values of k_mK_s give the free energies of transfer of substituents from the aqueous phase in the ground state to the micellar phase in the transition state.

The comparison of the micelle- and non-micelle-catalysed hydrolysis of penicillin derivatives is given in the Table. The data refer to 0.05M-sodium hydroxide and, as shown previously,[2] the apparent rate enhancements would be greater at lower hydroxide ion concentration. The binding constants are related to the Hansch π-substituent constant[17] for the 6-alkyl side chain as shown in Figure 4, using a constant π increment

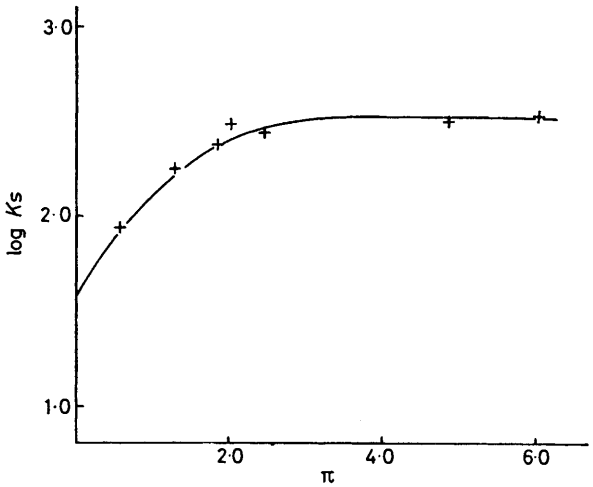


FIGURE 4 The equilibrium constants for binding of alkylpenicillins to micelles of CTABr as a function of the Hansch π-parameters

of 0.6 per additional methylene.[18] This non-linear relationship is reflected in an apparent decrease in the free energy of transfer of a methylene unit from water to the micelle with a maximum value of 0.48 kcal mol^{-1} * for transfer in the ground state and a maximum of 0.71 kcal mol^{-1} for transfer in the transition state.

It has been estimated[19] that the free energy change for the complete transfer of a single methylene unit from water to the micellar phase is 0.65 kcal mol^{-1}, which corresponds to a maximum rate or equilibrium difference of 3 at 25 °C. The free energy of transfer of a methylene group from water to a non-polar liquid is *ca.* 1.0 kcal mol^{-1},[20] and that to an enzyme from 2.1—3.8 kcal mol^{-1}.[21] An average value of 0.93 kcal mol^{-1} has been estimated from the tetradecyltrimethylammonium chloride-catalysed hydrolysis of 4-nitrophenyl acetate and hexanoate,[9] but one of 0.63 kcal mol^{-1} for the CTABr-catalysed hydrolysis of 4-nitrophenyl esters from inhibition binding constants which decreases from 0.75 kcal mol^{-1} for acetate–propionate to 0.57 kcal mol^{-1} for pentanoate–hexanoate.[22] The relative second-order rate constants for this micelle-catalysed reaction give a

* 1 cal = 4·184 J.

value of 0.45 kcal mol^{-1} per methylene varying from 0.26 to 0.52 kcal mol^{-1} but show no apparent trend with increasing chain length.[22] The carboxylate-catalysed iodine oxidation of diethyl sulphide in sodium dodecyl sulphate micelles gives an average value of 0.55 kcal mol^{-1} per methylene but this reflects an increase in rate of 1.9 ± 0.6 per methylene for acetate to pentanoate but 5.9 for pentanoate to hexanoate.[23] The equilibrium constants for imine formation from retinal and long chain amines in Triton X-100 increase uniformly for C_2—C_{10} amines, corresponding to 0.65 kcal mol^{-1} per methylene group, but are constant beyond C_{12} amines.[24] This may be contrasted with an average value of 0.31 kcal mol^{-1} per methylene for the rate of addition of cyanide to *N*-alkylcarbamoylpyridinium cations in CTABr micelles resulting in a value of 0.54 kcal mol^{-1} for C_8—C_{10} which smoothly decreases to 0.07 kcal mol^{-1} for C_{14}—C_{16}.[25] Similarly, the sodium lauryl sulphate catalysed formation of oximes from alkanones increases with increasing chain length from propan-2-one to heptan-2-one, giving a free energy of transfer of 0.16 kcal mol^{-1} per methylene, but then becomes independent of chain length.[26]

It appears that the observation of a saturation phenomenon with respect to increasing hydrophobicity of the substrate depends upon the structure of the rest of the substrate. In any case, the free energy of transfer of a methylene group from water to a micelle is considerably less than that available upon transfer to an enzyme. This presumably results from the 'loose' interactions between the micelle, composed of several molecules of detergent separated by their van der Waals radii, and the substrate compared with the 'tight' interactions available from the substrate molecule and one molecule of enzyme, composed of many atoms closely packed together.[21]

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