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MICELLAR CATALYSIS OF THE INTRAMOLECULAR AMINOLYSIS OF THE β-LACTAM ANTIBIOTIC CEPHACLOR

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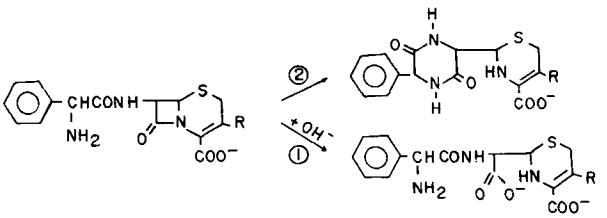
The rate of intramolecular degradation of cephaclor, involving the attack of the amino group at C-17 (N-18) on the β-lactam carbonyl, was increased up to 50-fold by neutral (polyoxyethylene-23 lauryl ether; Brij) and zwitterionic [3-(*N*-dodecyl-*N*, *N*-dimethylammonium)propane 1-sulphonate; SDP) micelles. The rate of OH^- attack on cephaclor was increased 2–3-fold by Brij and SDP micelles. In the absence of micelles the rate of intramolecular degradation of cephaclor increased by up to 2-fold by addition of organic solvents. Distance calculations, based on the crystal structure of the antibiotic, showed that the intramolecular degradation can only proceed in a conformation involving a *cis*-amide bond (N-14—C-15). Micellar catalysis of the intramolecular degradation process was proposed to be due to the stabilization of the reactive conformation.

INTRODUCTION

Detergents, widely used in pharmaceutical preparations, can affect drug stability and control rates and mechanisms of drug decomposition.[1] The widespread use of β-lactam antibiotics makes the study of their degradation of special importance.[2] The mechanism of β-lactam ring opening is both chemically interesting and informative with regard to antibiotic efficiency.[3] The therapeutic action of β-lactam antibiotics arises from the irreversible acylation of a key enzyme in the metabolic pathway leading to the synthesis of the bacterial cell wall.[4] The bactericidal efficiency of these antibiotics is related to the reactivity of the β-lactam carbonyl group.[5] The degradation pathways of β-lactam antibiotics include OH^- attack on the β-lactam ring and, in the case of cephalosporins, but not penicillins,[6] intramolecular aminolysis[7] (Scheme 1).

Controlled studies of micellar effects on the rate of pathway 1 (Scheme 1) have shown that positively charged micelles accelerate OH^- attack mainly by concentrating reagents.[1d] We have recently demonstrated that the rate of intramolecular degradation of cephaclor



Scheme 1

(pathway 2, Scheme 1) is catalysed by positively charged hexadecyltrimethylammonium bromide (CTAB) micelles.[1d]

Here we show that the rate of intramolecular degradation of cephaclor is increased up to 50-fold by micelles formed by neutral and zwitterionic detergents. The micellar-induced rate increase of the bimolecular OH^- attack on cephaclor by the same micelles was substantially smaller. Micellar-modified rates were unaffected by increasing buffer concentration. In homogeneous aqueous solution solvent addition increased the reaction rate by, at most, 1·5-fold. Calculations of the distance between the attacking N and the β-lactam carbonyl showed that cephaclor can only

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reach a reactive conformation in the *cis* form of the N-14—C-15 amide bond. Micellar effects on the intramolecular degradation of cephaclor are probably due to the stablization of a reactive conformation of the antibiotic in the micellar surface.

EXPERIMENTAL

Standard-grade cephaclor (**I**), grade I (lot P63370, Bulk Lot K07-3R-127) was kindly provided by Eli Lilly (Brazil). Stock solutions were prepared daily and maintained at 4 °C. Analytical-reagent grade polyoxyethylene-23 lauryl ether (Brij) and 3-(*N*-dodecyl-*N*, *N*-dimethylammonium)propane 1 sulphonate (SDP) were obtained from Sigma (St. Louis, MO, U.S.A.) Tris [tris(hydroxymethyl)aminomethane] (Carlo, Erba, Milan, Italy) and MOPS (4-morpholinopropanesulphonic acid) (Aldrich Chemical, Milwaukee, WI, U.S.A.) were used as received. Dioxane (E. Merck, Darmstadt, F.R.G.); dimethylformamide (DMF) (Fisher Scientific, Pittsburgh, PA. U.S.A.) and dimethyl sulphoxide (DMSO) (E. Merck) and ethanol (Merck, Brazil) were purified by standard procedures. Deionized, doubly glass-distilled water was used throughout. All other reagents were of analytical-reagent grade or better.

The hydrolysis of **I** was followed in a Beckman DU-7 spectrophotometer at 30 °C by measuring the decrease in absorbance of the unreacted antibiotic at 262 nm. First-order rate constants (k_ψ), calculated from ln(absorbance) versus time functions using a linear regression program on a personal microcomputer, are the averages of at least three determinations. All reactions followed first-order kinetics for at least four half-lives. The pK_a of the amino group of **I**, with or without added solvent, was determined from the variation of the absorbance at 237 nm[1d] with pH using a Digimed (São Paulo, Brazil) DMPH-3 pH meter. The pH electrode was calibrated in the presence of solvent at the nominal pH of the standard buffer. Kinetic data fitting was performed using a pseudo-phase model (PPM)[8] and atomic distances were calculated using a Molecular Model program[9] on a personal microcomputer by using x-ray crystallographic data for cephaloglycin.[10]

RESULTS

Degradation of cephaclor in aqueous solution at pH 7·8 occurs predominantly by an intramolecular pathway.[7] The spectral, kinetic and structural differences between the intra- and inter-molecular degradation pathways have been described.[7b,11] The rate of intramolecular degradation of cephaclor increased markedly on addition of both SDP and Brij (Figures 1 and 2). The observed rate constant (k_ψ) increased with increasing detergent concentration up to a plateau (Figures 1 and 2). This rate–detergent profile is typical of micellar-modified unimolecular reactions where the rate constant in the micelle (k_m) is higher than that in the aqueous phase (k_w).[12] At 0·08 M Brij or SDP, increasing the buffer concentration from 0·01 to 0·08 M (pH 7·8, constant ionic strength 0·08 M) did not change the reaction rate.

The effect of micelles on these reactions was analysed quantitatively using a pseudo-phase model where the variation of k_ψ with detergent can be expressed as[8,12]

$$k_\psi = k_w I_f/I_T + k_m I_b/I_T \quad (1)$$

where the subscripts f, b and T refer to the concentrations of free, bound and total cephaclor, respectively. The distribution of cephaclor between the micelles and the intermicellar aqueous phase can be expressed in terms of a substrate–micelle association constant (K_s):

$$K_s = I_b/(I_f C_D) \quad (2)$$

The concentration of micellized detergent (C_D) represents the total analytical concentration of added detergent (C_T) minus the critical micelle concentration (CMC). From equations (1) and (2) one obtains a linearized expression for the variation of k_ψ with detergent concentration:

$$1/(k_\psi - k_w) = 1/(k_m - k_w) + [1/(k_m - k_w)]K_s(1/C_D) \quad (3)$$

The linear plots are shown in Figures 1(B) and 2(B).

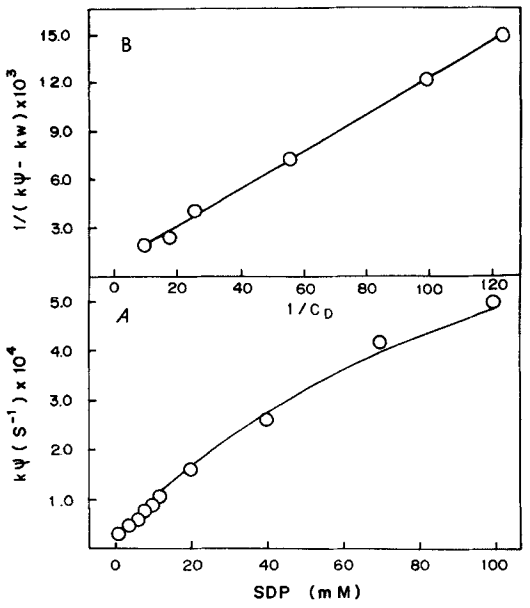

Figure 1. Effect of SDP on the rate of intramolecular degradation of **I**. $2{\cdot}5 \times 10^{-5}$ M cephaclor, 0·01 M MOPS buffer, pH 7·8, 0·01 M total ionic strength. (A) Effect of SDP; line was calculated (see text). (B) Data in (A) plotted according to equation (3)

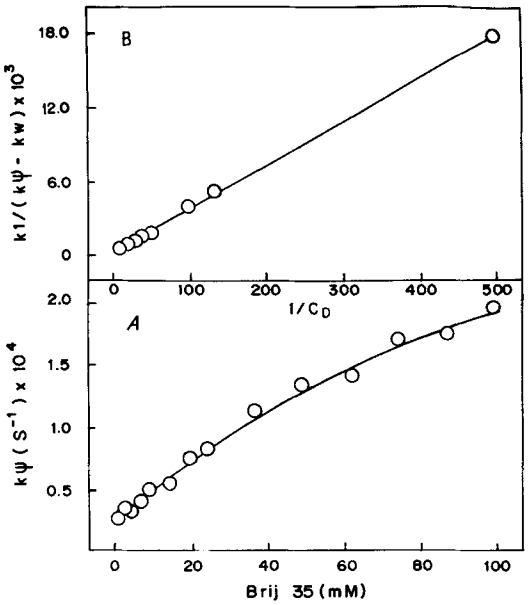

Figure 2. Effect of Brij on the rate of intramolecular degradation of **I**. $2{\cdot}5 \times 10^{-5}$ M cephaclor, 0·01 M MOPS buffer, pH 7·8, 0·01 M total ionic strength. (A) Effect of Brij; line was calculated (see text). (B) Data in (A) plotted according to equation (3)

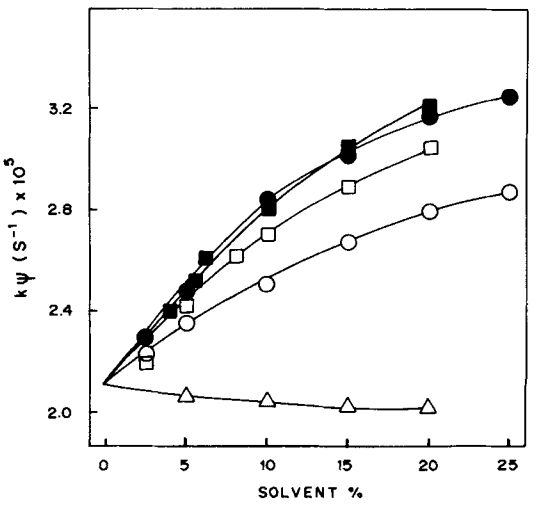

Figure 3. Solvent effects on the rate of intramolecular degradation of **I**. $2{\cdot}5 \times 10^{-5}$ M cephaclor, 0·01 M MOPS buffer, pH 7·8, 0·01 M total ionic strength. ■, DMF; •, DMSO; □, dioxane; ○, acetonitrile; △, ethanol

The calculated values of K_s and k_m are presented in Table 1. The lines in Figures 1(A) and 2(A) were calculated [equation (2)] using the values of K_s and k_m given in Table 1. For unimolecular reactions the k_m/k_w ratio can be taken as a measure of the catalytic efficiency of the micelle.[12] The k_m/k_w ratios for the unimolecular decomposition of cephaclor were 20 and 46 for Brij and SDP micelles, respectively (Table 1).

In several cases, particularly for some intramolecular reactions, micellar effects have been attributed to the change in free energy of activation resulting from the transfer of the reactant to a medium of lower dielectric constant.[13] Micellar catalysis can be lower than that expected from solvent effects in the same reaction and the effective dieletric constant at the reaction site.[14] The rate of intramolecular degradation of cephaclor increased with addition of DMSO, dioxane, DMF and acetonitrile to the aqueous solution and decreased

Table 1. Parameters used for curve fitting

Surfactant	pH	K_s (l mol^{-1})	k_m ($s^{-1} \times 10^4$)	k_w ($s^{-1} \times 10^4$)	k_m/k_w
SDP	7·8	8·86	9·91	0·211	46·5
	10·8	9·02	13·20	5·45	2·4
Brij	7·8	6·96	4·38	0·211	20·5
	11·0	7·05	10·80	7·20	1·5

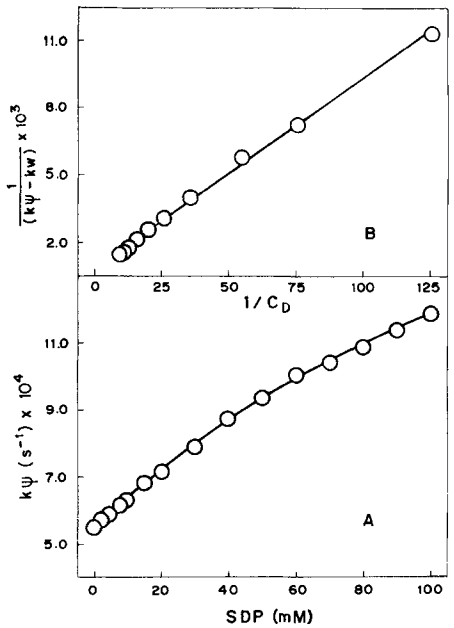

Figure 4. Effect of SDP on the rate of intramolecular degradation of **I**. $2{\cdot}5 \times 10^{-5}$ M cephaclor, 0·01 M MOPS buffer, pH 7·8, 0·01 M total ionic strength. (A) Effect of SDP. (B) Data in (A) plotted according to equation (3)

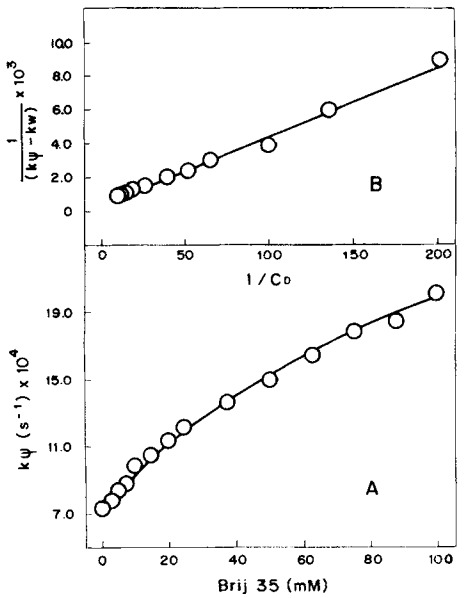

Figure 5. Effect of Brij on the rate of intramolecular degradation of I. $2{\cdot}5 \times 10^{-5}$ M cephaclor, 0·01 M MOPS buffer, pH 7·8, 0·01 M total ionic strength. (A) Effect of Brij. (B) Data in (A) plotted according to equation (3)

slightly with the addition of ethanol (Figure 3). The maximum solvent effects on the reaction rates were much lower than those obtained with micelles. Appropriate controls ensured that the moderate rate increases reflected solvent effects on the rate of intramolecular degradation rather than pK_a shifts.

At pH 10 or higher, the predominant degradation pathway of cephaclor is a bimolecular OH^- attack.[7,11] Brij and SDP micelles produced only a small enhancement of the rate of OH^- attack on cephaclor (Figures 4 and 5). The kinetics of the micelle-modified bimolecular reaction were also analysed using the pseudo-phase distribution model described above. The calculated values of distribution and rate constants are presented in Table 1. For the same detergent, the calculated values of the micelle–water distribution constants of cephaclor were similar for both intra- and inter-molecular degradation pathways (Table 1). With both detergents the ratio of rate constants in the micellar and aqueous pseudo-phases was much larger for the intramolecular reaction (Table 1).

DISCUSSION

A major factor in the micellar rate enhancement of bimolecular reactions is reagent concentration, the second-order rate constants in the micelle being of the same order of magnitude as those in aqueous solution.[12,15,16] The rate of alkaline decomposition of cephaclor was increased by Brij and SDP micelles and, as with similar reactions in the same micelles,[17] the (calculated) k_m/k_w ratios lie between 1·5 and 2·4.

For unimolecular reactions, increases in the k_m/k_w ratios can be equated with micellar catalysis.[1d,12] The intramolecular degradation of cephaclor is catalysed by positively charged micelles[1d] and was also catalysed by zwitterionic and neutral micelles by factors ranging from 20 to 50.

The mechanism of intramolecular aminolysis involves proton-transfer steps that may become rate limiting.[18] The rate of intramolecular aminolysis was increased by CTAB, SDP and Brij and it would be an extreme coincidence that positive, zwitterionic and neutral micelles decreased the energy involved in proton transfers by similar magnitudes. Moreover, although this reaction is catalysed by buffers in aqueous solution,[11a,19] we did not detect buffer catalysis in the micelle-modified intramolecular degradation of cephaclor.

The apparent dielectric constants at the interface of micelles are not substantially affected by micellar charge.[12] Thus, the reaction rate could be similarly affected in all three micellar types by the decrease in the local dielectric constant, as described for solvent-sensitive unimolecular reactions.[13] Ethanol, a solvent used to mimic micellar effects,[12] slightly decreased the reaction rate. The rate of intramolecular degradation of cephaclor increased with the addition of other organic solvents, but the maximum rate enhancement was *ca* 2-fold (Figure 3). Thus, a dielectric constant effect seems not to be the source of the observed catalysis.

In the conformation corresponding to the crystal structure of cephaclor the N-18—C-8 distance was 5·71 Å [Figure 6(a)]. Single and combined rotations of the bonds R_1 and R_3 [Figure 6(a)] permitted a maximum approximation of 4·55 Å. These latter distances are much too large to allow reaction.[20] Intramolecular aminolysis in cephalosporins proceeds by the attack of N-18 on the β-face of the β-lactam ring, i.e. *cis* to the amide bond.[5,6] The N-18 to C-8 distance in the *cis* conformation was 1·54 Å [Figure 6(b)]. At this distance descriptions of intramolecular reaction theory accept the possibility of bond formation.[20] The angle of approach of N-18 to the (β-face) carbonyl from the previously described *cis* conformation [Figure 6(b)] was 118°, close to the most favoured approach angle of nucleophiles attacking carbonyl groups (104°).[21] Hence, on several grounds, the most probable reactive conformation for the intramolecular attack is that shown in Figure 6(b). There are no data that permit us to confirm that the rate-limiting step for this reaction is the attack itself. In fact, in several well documented cases of aminolysis the attack is not rate limiting. However, in the case of β-lactam the relief of strain of the four-membered ring may make the attack rate limiting. Moreover, in the case of cephaclor the reactive conformation is separated from the crystal conformation by

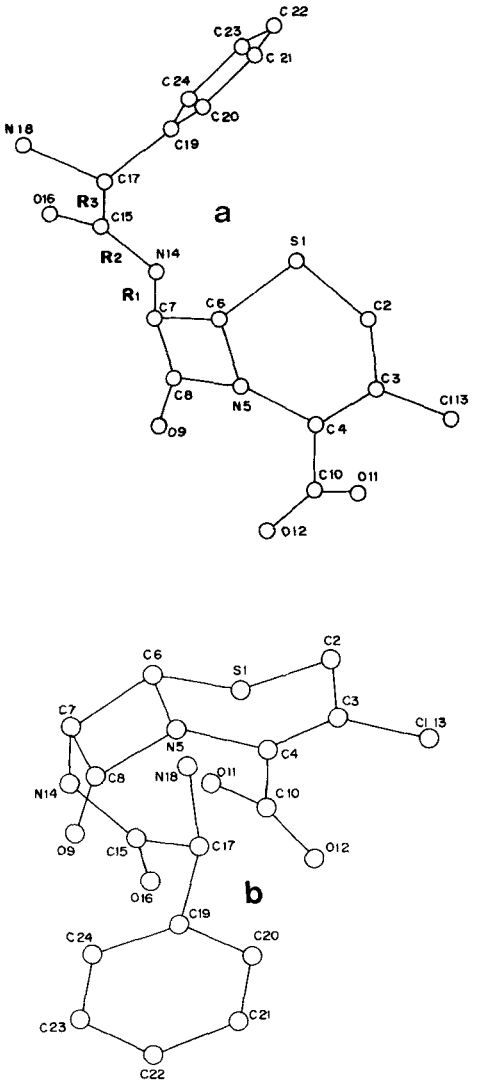

Figure 6. (a) Conformation of **I** in the crystal form. (b) Molecular structure of **I** in the reactive conformation (for intramolecular degradation)

several kilocalories (1 kcal = 4·184 kJ), since it could involve a *trans–cis* isomerization of a peptide bond.[22] Hence it is possible that the modest effective molarity[23] for the intramolecular aminolysis[18] is attributable to the energy barrier for the amide *trans–cis* isomerization

In several cases *cis–trans* amide isomerizations are affected by micelles.[24] We propose that micellar catalysis in the intramolecular aminolysis of cephaclor is determined by the stabilization of a particular conformation of the substrate.

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