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COMMUNICATIONS

Effect of surfactants on degradation of penicillins and cephalosporins in acidic medium

AKIRA TSUJI, MUNEAKI MATSUDA, ETSUKO MIYAMOTO, TSUKINAKA YAMANA*, *Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa* 920, *Japan*

It is well recognized that the acid stability of penicillins and cephalosporins is a factor affecting their oral absorption. The susceptibility of penicillins to acid-catalysed degradation is attributed to the intramolecular attack of the side-chain amide carbonyl upon the β-lactam moiety (for reviews, see Hou & Poole, 1971). The rate of cleavage of the β-lactam largely depends on the polar nature of the side chain (Doyle, Nayler & others, 1961).

We found that the acid degradation of penicillins was significantly affected in a variety of ways by the addition of surfactants. An anionic surfactant enhanced the rate, whereas both cationic and non-ionic surfactants markedly inhibited degradation, as the result of penicillin-micelle interactions. During the preparation of this communication a report of the catalytic effect of a cationic micelle on the degradation of cephalexin at neutral pH appeared (Yasuhara, Sato & others, 1977). The present paper describes surfactant effects on the degradation of β-lactam antibiotics in acidic medium.

The degradation of β-lactam antibiotics (0·5 to 5 mM depending upon their solubilities) was studied at 35° in hydrochloric acid solution, which was, unless otherwise stated, adjusted to 0·5 M ionic strength with potassium chloride. Penicillins were determined by the imidazole method developed by Bundgaard & Ilver (1972). Cefazolin was analysed by reversed-phase high-pressure liquid chromatography (h.p.l.c.), the conditions used were: instrument, JASCO FLC A-700 equipped with an ultraviolet detector (254 nm); column, 125 mm × 4·5 mm i.d., SC-01, 5 μm, ODS chemically bonded on the totally porous silica gel (JASCO, Tokyo, Japan); mobile phase, 10% acetonitrile, 90% 0·01 M ammonium acetate; flow rate, 1 ml min^{-1}; pressure 11·8 MNm^{-2}. All antibiotics studied followed pseudo-first-order kinetics, the rate constants, k_{obs} h^{-1}, were calculated by a least squares evaluation from the slopes of the plots of the residual concentration *vs* time.

As illustrated in Fig. 1, the pseudo-first-order rate constant for the degradation of propicillin at pH 1·6 was significantly increased (Fig. 1A) by the addition of an anionic surfactant, sodium lauryl sulphate (SLS), whereas it was decreased (Fig. 1B) by increasing the concentration of cationic or non-ionic surfactants, hexadecyltrimethyl ammonium bromide (CTAB) and polyoxyethylene-23-lauryl ether (POE), respectively. In all cases, the rate constants first increased or decreased rapidly and then approached a contant value above the critical micelle concentrations of the surfactants (cmc, see Table 1), suggesting the formation of penicillin-micelle complexes.

It is often possible to explain micellar catalysis or retardation by making certain simplifications and assuming that only one substrate molecule (penicillin) is incorporated into a micelle, and that the aggregation number, N, of the micelle is independent of the substrate. There will then be an equilibrium between substrate (S) in solution and that (MS) in the micelle (eqn 1)

$$\begin{array}{ccc} & \overset{K}{\rightleftharpoons} & \\ M + S & & MS \\ \downarrow k_o & & \downarrow k_m \\ P & & P \end{array} \quad \ldots \quad (1)$$

where k_o and k_m are the rate constants for the product formation in the bulk solution and in the micellar phase, respectively, and K is the binding constant for the substrate-micelle interaction (Fendler & Fendler, 1975).

The concentration of the micelles, [M], is given by

$$[M] = \frac{C_D - cmc}{N} \quad \ldots \quad (2)$$

where C_D is the total concentration of surfactant. The observed first-order rate constant is expressed as eqn 3

$$k_{obs} = \frac{k_o + k_m K[M]}{1 + K[M]} \quad \ldots \quad (3)$$

Combination of equations 2 and 3 and rearrangement gives

$$\frac{1}{k_o - k_{obs}} = \frac{1}{k_o - k_m} + \left(\frac{1}{k_o - k_m}\right)\left(\frac{1}{C_D - cmc}\right)\frac{N}{K} \quad \ldots \quad (4)$$

Equation 4 predicts that plots of $1/(k_o - k_{obs})$ or $1/(k_{obs} - k_o)$ against $1/(C_D - cmc)$ should give a straight line from which is should be possible to obtain k_m and K/N values.

* Correspondence.

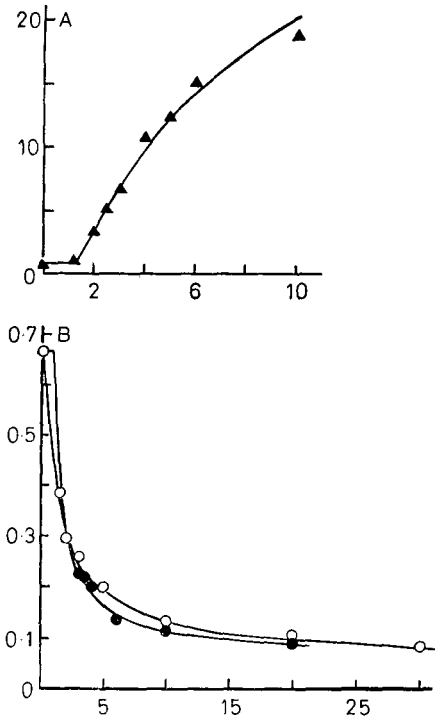

FIG. 1. Plots of the observed first-order rate constant, k_{obs} (h^{-1}) (ordinate) against A: the SLS concentration (▲); and B: the POE (○) and CTAB (●) concentrations, C_D (mM) (abscissa) for the acid degradation of propicillin at pH 1·6, 35° and ionic strengths A 0·03 M and B 0·5 M. The curves were calculated from eqns 2 and 3 and the parameters listed in Table 1.

Typical plots of equation 4 are shown in Fig. 2 for the degradation of several penicillins in the presence of POE. The values of k_o, k_m, and K/N for the various reaction systems are given in Table 1.

It was found that the kinetics for equation 3 held for the acid degradation of penicillins in the presence of all types of surfactants, as similarly observed for the neutral degradation of cephalosporins in the micellar solution of CTAB (Yasuhara & others, 1977).

As is apparent from Table 1, the K/N value for POE and CTAB increased with increasing lipophilic character of the penicillins, as expressed in terms of octanol-water partition coefficients, P. This suggests that hydrophobic binding is involved in the interaction between the cationic and non-ionic micelles and undissociated penicillins and presumably these strong interactions protect the β-lactam ring sterically and/or electrostatically from the intramolecular and nucleophilic attack of the side-chain amide carbonyl oxygen. In cationic and non-ionic micelles the penicillins can be stabilized by a factor of about 4 to 12 depending on their lipophilicity. On the other hand, it is possible that because of the localized hydrogen-ion activity surrounding the negatively charged micelle, the anionic micellar state leads to a increase in the rate of degradation.

Table 1. *Parameters*[a] *interpreting the effects of surfactants on the acid degradation of β-lactam antibiotics at pH 1·6, 35° and ionic strength 0·5* M.

Antibiotics and surfactants	k_o h^{-1}	k_m h^{-1}	K/N M^{-1}	log P[b]
Propicillin				
POE	0·678	0·053	683	2·70
CTAB		0·065	1265	
SLS[c]	0·678	39·1	114	
Phenethicillin				
POE	0·607	0·083	338	2·20
CTAB		0·074	695	
Penicillin V				
POE	0·490	0·078	311	1·95
CTAB		0·048	554	
Penicillin G				
POE	17·6	4·35	178	1·70
CTAB[d]	6·87	1·22	250	
Cefazolin				
POE	0·114	0·108[e]		0·39[f]
CTAB		0·078[e]		
SLS[c]		0·110[e]		

a Calculated according to eq. 4 by the least squares method. The values of cmc used for the calculations are 0·08 mM for POE, 0·92 mM for CTAB, and 1·35 mM for SLS (Fendler & Fendler, 1975).
b Partition coefficient between n-octanol and water reported previously (Tsuji & others, 1977).
c Ionic strength 0·03 M.
d pH 2·1.
e k_{obs} (h^{-1}) at 10 mM surfactant concentration.
f Yamana & others (1977).

In contrast to the penicillins, the acid degradation of cefazolin, a relatively acid-unstable cephalosporin (Yamana & Tsuji, 1976) was not influenced by the

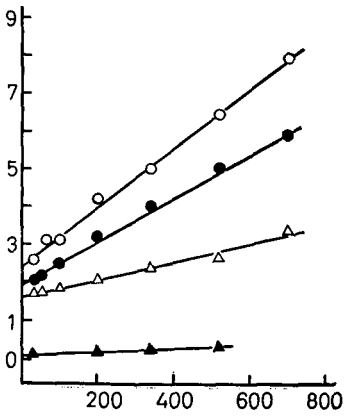

FIG. 2. Double reciprocal plots according to eqn 4 for the acid degradation of propicillin (△), phenethicillin (●), penicillin V (○) and penicillin G (▲) in the presence of POE at pH 1·6, 35° and ionic strength 0·5 M. Ordinate: $1/(k_o-k_{obs})$ (h). Abscissa: $1/(C_D-cmc)$ (M^{-1}).

presence of any type of surfactant (Table 1). This suggests that cefazolin is not sufficiently bound to the micelles, because of its lower hydrophobicity (Yamana, Tsuji & others, 1977), to prevent or accelerate the degradation.

The interaction between anionic forms of penicillins, with lower lipophilicity (Tsuji, Kubo & others, 1977) and non-ionic micelles was significantly decreased. The K/N value at pH 6·5 for propicillin in POE (10 mM) micellar solution was found, by the dynamic dialysis method (Meyer & Guttman, 1968), to be 42 M^{-1}, indicating that about 70% of the penicillin anions exist in the free form.

A use for the non-toxic and non-ionic surfactant POE to improve oral bioavailability of acid-labile and poorly absorbed β-lactam antibiotics is proposed because there is: (1) an enhancement of gastro-intestinal absorption (Davis, Pfeiffer & Quay, 1970), (2) a stabilizing ability towards acid degradation, and (3) a good release of the substrate from the micelle at intestinal pH.

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Increased binding of [^{3}H]apomorphine in caudate membranes after dopamine pretreatment *in vitro*

C. MCMANUS, E. J. HARTLEY, P. SEEMAN*, *Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8*

Most patients with Parkinson's disease being treated with L-dopa develop an increased frequency of abnormal involuntary movements during therapy (Barbeau, 1976). This progressively deteriorating response may be attributed to either the natural pathology of the disease (Meunter, Sharpless & others, 1977) or to an L-dopa-induced process of unknown origin (Martres, Costentin & others, 1977).

Although long-term exposure of β-adrenergic catecholamines generally results in tachyphylaxis or desensitization (Makman, 1971; Kebabian, Zatz & others, 1975; Franklin & Twose, 1976; Mukherjee & Lefkowitz, 1977), long-term administration of dopamine-mimetic drugs to animals sometimes produces 'behavioural facilitation' (Segal & Mandell, 1974; Friedman, Rotrosen & others, 1975; Klawans & Margolin, 1975; Short & Shuster, 1976; Kilbey & Ellinwood, 1977; but see the work of Martres & others, 1977 on single doses of apomorphine).

To investigate one possible molecular mechanism of this facilitation or sensitization induced by dopamine-mimetic drugs, we tested the effects of prolonged exposure (*in vitro*) of dopamine on the dopamine/neuroleptic receptors in the caudate nucleus of the calf.

Preparation of calf caudate homogenates. The experiments were done on crude homogenates of calf caudate, prepared as described by Seeman, Lee & others (1976, a,b). The homogenized tissues were finally resuspended in 10 vol of ice cold buffer (15 mM tris-HCl, pH 7·4, 5 mM Na_2EDTA, 1·1 mM ascorbic acid and 12·5 μM nialamide), incubated at 37° for 60 min, and stored frozen at −20° for several days or weeks until used further.

Pretreatment procedure. Before testing for the binding of [^{3}H]apomorphine, [^{3}H]haloperidol, ^{3}H-WB-4101 [^{3}H]2(*N*[2,6-dimethoxyphenoxyethyl])amino-methyl-1,-

* Correspondence.