Tetrahedron Letters No. 44, pp 3851 - 3854, 1977. Pergamon Press. Printed in Great Britain.

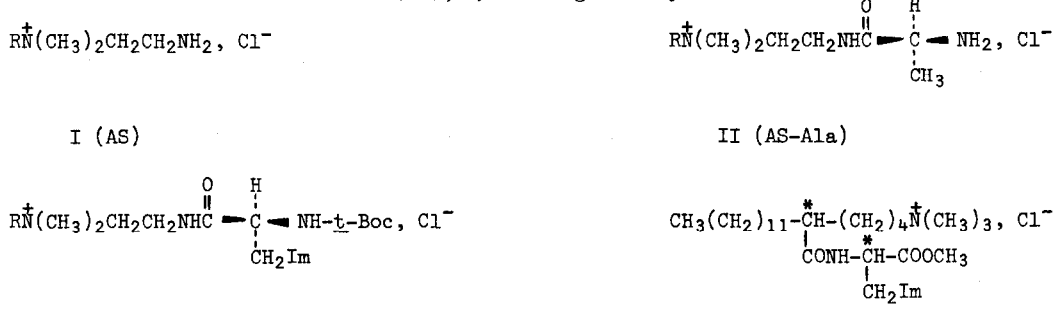
NEW AMINO ACID-FUNCTIONALIZED SURFACTANTS; PREPARATION AND CATALYTIC PROPERTIES

Robert A. Moss,* Thomas J. Lukas, and Robert C. Nahas
Wright and Rieman Laboratories, Department of Chemistry,
Rutgers, The State University of New Jersey,
New Brunswick, New Jersey 08903

(Received in USA 15 August 1977; received in UK for publication 16 September 1977)

Functionalized surfactant micelles are being increasingly studied as enzyme analogues and as catalysts in their own right.[1-4] The twin goals of good modeling and catalytic efficacy point to amino acid-functionalized surfactants as immediate targets. Thus, long-chain acyl derivatives of histidine,[5] cysteine,[6] serine,[5d] tyrosine,[5d] and arginine,[5d] generally comicellized with cetyltrimethylammonium (CTA) bromide, have been used as esterolysis catalysts under basic conditions. However, ionization of the carboxyl groups of these micellar reagents suppresses ionization of their less acidic, potentially nucleophilic confunctionalities, and also partially neutralizes the positively charged CTA head groups. Both factors mitigate catalytic potential in weakly basic media. Alternatively, Brown and Bunton have coupled L-histidine methyl ester to 5-carboxyheptadecyltrimethylammonium chloride.[7a,b] In the resulting (micellized) reagent, discussed below, the histidine probably resides in an hydrocarbon-like region.

We now report a new approach to the preparation of amino acid-functionalized surfactants, and summarize relevant catalytic properties toward p-nitrophenyl acetate (PNPA) and D- or L-N-acetylphenylalanine p-nitrophenyl ester (N-AcPhe-PNP). Quaternization of N,N-dimethyl-N'-acetylethylene diamine with cetyl chloride, followed by deacetylation with 6N aq. HCl, gave I·HCl, which could be converted to the free aminosurfactant (AS), I,[8,9] using Amberlyst A21 beads.



I (AS) II (AS-Ala)

III (AS-His-Boc) IV

(R = n-$C_{16}H_{33}$, Im = 4-imidazolyl)

N-t-Boc-(L)-alanine was coupled (DCCI, CH_2Cl_2, 0°) to polystyrene-bound 1-hydroxybenzotriazole,[10a] affording an active ester which was reacted with I (CH_2Cl_2, 25°, 45 min.), affording after deprotection (HCl/HOAc) AS-Ala, II,[9,11] as the hydrochloride. Similarly, N^{α}, N^{im}-di-t-Boc-(L)-

Table I. Kinetics of Hydrolyses of PNPA Catalyzed by Surfactant Micelles.[a]

Catalyst	k_{ψ}^{max} (sec^{-1})[b]	k_{cat} (1/Mol-sec)[c]	k_{cat}^{rel}
CTACl[d]	0.00010 [1.3]	0.0077	1.0
AS[d]	0.026 [3.7]	0.70	91.
AS-Ala[d]	0.0053 [1.5]	0.35[e]	45.
CTACl[f]	0.00019 [1.3_5]	0.014	1.0
AS-Ala[f]	0.010 [3.1]	0.32	23.
AS-His-Boc[f]	0.029 [1.6]	1.8	130.
16-Im[g]	0.20 [4.0]	5.0	360.

[a]See text for catalyst structures and ref. 13 for conditions. [b]Values in [] are concentrations (M x 100) at which k_{ψ}^{max} was determined. [c]$k_{cat} = k_{\psi}^{max}/[surfactant]$. [d]Tris buffer.[13] [e]k_{cat} = 0.47 1/mol-sec, corrected to 100% free NH_2 form. Note that the value given for k_{ψ} of AS-Ala in tris buffer is not a maximum value; comparison with AS-Ala phosphate data, however, suggests that k_{cat}^{tris} would not substantially change, were it derived from k_{ψ}^{max}. [f]Phosphate buffer.[13] [g]0.01 M phosphate buffer; see ref. 3.

Table II. Kinetics of Hydrolyses of N-AcPhe-PNP[a]

Catalyst	10^3[Cat.], M	$k_{\psi}^{D,max}$ ($s.^{-1}$)	$k_{\psi,}^{L,max}$ ($s.^{-1}$)	k_{cat}^{L} 1/Mol-sec[b]	k_{cat}^{rel}
CTACl	4.0		0.089	22.	1.0
AS-(L)-Ala	2.6	0.039	0.044	17.	0.77
AS-(L)-His-Boc	4.3	0.25	0.26	60.	2.7
16-Im	2.5	2.3	2.3	920.	42.

[a]See text for catalyst structures; all experiments were done in phosphate buffer.[13] [b]See Table I, note c.

histidine was coupled[10a,b] to I, whence deprotection (NH_3, CH_3OH) of the imidazole gave AS-His-Boc, III.[9,12]

Hydrolyses[13] of PNPA catalyzed by micellar I-III, CTACl, and N-cetyl-N,N-dimethyl-N-4-methylimidazolylammonium chloride (16-Im)[3] were followed spectrophotometrically at 400 nm (liberation of p-nitrophenoxide). Variation of [surfactant] afforded rate constant-[surfactant] profiles from which were obtained the k_{ψ}^{max} values in Table I. Similarly, we determined k_{ψ}^{max} values for hydrolyses[13] of (D) or (L)-N-AcPhe-PNP; cf., Table II.

The amino-functionalized surfactants, I and II, are 45-90 times more effective than CTACl at cleaving PNPA at pH 8 (Table I). Comparison with previous results[3] also reveals I to be >5 times superior to $n\text{-}C_{16}H_{33}\overset{+}{N}(CH_3)_2CH_2CH_2OH$, Cl^- (16-OH) under comparable conditions, reflecting the essentially complete availability of I (pk_a~6.05) in the nucleophilic NH_2 form (at pH 8) whereas, under these conditions, 16-OH (pk_a~10.5)[14] is only marginally converted (<1%) to the strongly nucleophilic $16\text{-}O^-$ form.[14]

On the PNPA scale, AS-His-Boc is 5.7 times more reactive than AS-Ala, but 2.8 times less reactive than the simple imidazolyl surfactant, 16-Im. As with the latter,[4] cleavage of PNPA by AS-His-Boc involves the transient intermediacy of a N-acetylimidazole, detectable at 247.5 nm. However, although the principal nucleophile in 16-Im-PNPA reactions must be the anionic imidazole moiety,[3] preliminary evidence implicates the less nucleophilic, neutral imidazole form in the AS-His-Boc-PNPA reactions: comparing AS-His-Boc with 16-Im, the ratios k_{H_2O}/k_{D_2O} [pH(D) = 8.0] are 1.4 vs. ~2.3,[15] and the slopes of log k vs. pH (7.0-9.0) are ~0.2 vs. 0.76.[15] The greater importance of the anionic imidazole moiety of 16-Im probably reflects a lower $pk_{a(2)}$ for this group, relative to AS-His-Boc, due to the smaller separation of the Im and quaternary nitrogen in the former surfactant.

AS-Ala and AS-His-Boc are good catalysts for the cleavage of PNPA, but Table II reveals that they lack stereoselectivity in micellar hydrolyses of N-AcPhe-PNP substrates. In terms of K/N[16] and k_{ψ}^{max}, AS-His-Boc and IV[7a] are comparable,[17] but the latter gave $k_{\psi}^{L,max}/k_{\psi}^{D,max}$ ~3 with N-AcPhe-PNP in 0.02M phosphate at pH 7.35.[7a] It was suggested that "attachment of the reactive group to the alkyl chain rather than to the head group may well be advantageous" [in enantioselectivity]. This idea could account for the greater enantioselectivity of IV, although recent work has shown that only one diastereomer of IV is enantioselective,[18] suggesting greater complexity in the relation between stereoselectivity and surfactant structure.

More importantly, N-AcPhe-PNP is very reactive and possibly a poor substrate for the demonstration of micellar enantioselectivity; k_o = 6.2 x 10^{-3} $sec.^{-1}$, more than 100 times greater than k_o for PNPA (1.8 x 10^{-5} $sec.^{-1}$)[3] under comparable conditions in phosphate buffers.[13] Furthermore, although simple micellar enhancement of substrate cleavage (i.e., $k_{\psi}^{max,CTACl}/k_o$) is similar for N-AcPhe-PNP and PNPA (14 vs. 11), surfactant functionalization has a minimal effect on micellar cleavage of the former substrate (cf., Table II, k_c^{rel} values). Whereas, toward PNPA, AS-His-Boc is 130 times more effective than CTACl (cf., Table I), toward N-AcPhe-PNP it is only 2.7 times more effective. And, AS-Ala, kinetically superior to CTACl by a factor of >45 on the PNPA scale, is actually less effective on the N-AcPhe-PNP scale. This suggests that, at least under the present micellar conditions, N-AcPhe-PNP is so reactive that the actual extent of participation of surfactant functionality is

small relative to that of micelle-bound hydroxide ions. Greater enantioselectivity may be observed with less reactive chiral substrates, in less hydrophilic micellar regions. These and other alternatives are under study.

Acknowledgments. We thank the Public Health Service (Research Grant CA-14912 from the National Cancer Institute) and the National Science Foundation for financial support. The stopped-flow spectrometer was purchased under P.H.S. grant RR-7058-09 to Rutgers University.

References and Notes

(1) J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems," Academic Press, New York, N. Y., 1975, pp. 169ff.

(2) For leading references, see ref. 3, notes (5) and (6), and ref. 4, notes (5)-(9).

(3) R. A. Moss, R. C. Nahas, S. Ramaswami, and W. J. Sanders, Tetrahedron Lett., 3379 (1975).

(4) R. A. Moss, R. C. Nahas, and S. Ramaswami, J. Amer. Chem. Soc., 99, 627 (1977).

(5) (a) C. Gitler and A. Ochoa-Solano, ibid., 90, 5004 (1968); (b) R. G. Shorenstein, C. S. Pratt, C-J. Hsu, and T. E. Wagner, ibid., 90, 6199 (1968); T. E. Wagner, C-J. Hsu, and C. Pratt, ibid., 89, 6366 (1967); (c) P. Heitmann, R. Husung-Bublitz, and H. J. Zunft, Tetrahedron, 30, 4137 (1974); (d) T. Inoue, K. Nomura, and H. Kimizuka, Bull. Chem. Soc. Japan, 49, 719 (1976).

(6) P. Heitmann, Eur. J. Biochem., 5, 305 (1968).

(7) (a) J. M. Brown and C. A. Bunton, Chem. Commun., 969 (1974); (b) J. M. Brown, C. A. Bunton, and S. Diaz, ibid., 971 (1974).

(8) Critical micelle concentration (cmc) = 3.0 ± 0.2 x 10^{-4} M in 0.05 M tris buffer, μ = 0.05 (KCl), pH 8.0 (surface tension); pk_a ~6.05 (pH-rate profile with PNPA).

(9) Satisfactory spectral and analytical characterizations were obtained for I·HCl and III. AS-Ala·HCl was very hygroscopic, and a satisfactory analysis for C could not be obtained, although separate analyses bracketed the theoretical value (±1%); satisfactory H and N analyses were obtained.

(10) (a) R. Kalir, A. Warshawsky, M. Fridkin, and A. Patchornik, Eur. J. Biochem., 59, 55 (1975). This method of coupling occurs in high yield and with very little racemization; reported applications include Ala and His. (b) His could be cleaved (hot 6N HCl) from AS-His-Boc and recovered with no significant loss of optical activity.

(11) Cmc = 1.6 x 10^{-4} M in 0.02 M PO_4 buffer, μ = 0.05, pH 8.0 (surface tension); pk_a ~7.5 (pH-rate profile with PNPA; $[\alpha]_D^{24}$ + 3.7° (c = 1.76, CH_3OH).

(12) Cmc = 1.1 x 10^{-4} M (conditions as in ref. 11); pk_a ~5.8 (pH-rate profile with PNPA); $[\alpha]_D^{25}$ + 6.8° (c = 0.11, 0.02 M PO_4 buffer, pH 8.0).

(13) pH 8, 0.02 M phosphate or tris buffers, μ = 0.05 (KCl), 25° C., [substrate] = 2.0 x 10^{-5} M.

(14) K. Martinek, A. A. Levashov, and I. V. Berezin, Tetrahedron Lett., 1275 (1975).

(15) R. C. Nahas, unpublished observations.

(16) Cf., ref. 1, pp. 86ff.

(17) For AS-His-Boc and N-Ac-(L)-Phe-PNP, K/N = 900; the comparable datum is 720 for IV ($k_{\psi}^{L,max}$ = 0.39 $sec.^{-1}$).

(18) Private communication from Dr. J. M. Brown, University of Oxford.

(19) The effect of functionalization seems to be more marked in the reaction of 16-Im and N-AcPhe-PNP (Table II), which may indicate a particular sensitivity of this substrate toward the anionic[3] imidazole moiety.