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MICELLAR EFFECTS UPON THE REACTIONS OF AMINO ACIDS AND THEIR DERIVATIVES WITH 2,6-DINITRO-4-TRIFLUOROMETHYLBENZENE SULFONATE ION[1]

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Abstract—In the absence of surfactants 2,6-dinitro-4-trifluoromethylbenzene sulfonate ion (1) and 2,4-dinitrofluorobenzene (DNF) have similar reactivities towards glycinate and glycylglycinate ions, but α-substituents hinder reactions with 1 but not with DNF, and hydroxide ion is relatively unreactive towards 1. Cationic micelles of cetyltrimethylammonium bromide (CTABr) strongly catalyze reactions of 1 with leucinate, phenylalaninate or α-phenylglycinate, but there is little catalysis of reactions of the more hydrophilic nucleophiles glycinate, and glycylglycinate ions, glycineamide and hydroxide ion, whereas the CTABr catalysis of reactions of DNF is less sensitive to the nature of the nucleophile. Rate enhancements by CTABr of the reactions of 1 are: glycinate 6(28); glycylglycinate 6(26); leucinate 93(34); phenylalaninate 740(104); α-phenylglycinate 247(65); glycineamide ~1(5·5); OH^- 3(60). (The values for DNF are in parentheses). The concentrations of CTABr necessary for catalysis of reactions of 1 are much less than for reactions of DNF. These observations suggest that 1 interacts very strongly with CTABr micelles. Added salt decreases the CTABr catalysis and anionic micelles of sodium lauryl sulfate do not affect reactions of 1 with glycinate or glycylglycinate.

Cationic micelles effectively catalyze the reaction of dinitrohalobenzenes with nucleophiles in aqueous solution,[2] and micellar catalysis and inhibition of the reaction of 2,4-dinitrofluorobenzene (DNF) with amino acids and peptides has been examined as a model for protein modification by DNF or other activated aromatic compounds.[3-5] Activated benzene sulfonates, being water soluble, are very useful agents for protein modification.[10] The reaction of 2,4,6-trinitrobenzene sulfonate ion with amino acid anions is cleanly second order,[10a,b] and Freedman and Radda showed that different reactivities of amino acid groups could be observed when this reagent is used for protein modification.[10b] We have examined the micellar catalysis of the reaction of 2,6-dinitro-4-trifluoromethylbenzene sulfonate ion (1) with a variety of amines, largely amino acid anions, and with hydroxide ion, because the surface of a micelle should have properties similar to that of a protein surface.[8] The micellar catalysis of the reaction of 1 with amino acid anions is sensitive to alkyl and aryl substituents on the amino acid, and we therefore examined some of these reactions with DNF for comparison.

The rate limiting step of aromatic nucleophilic substitution is generally addition,[11] and micellar effects upon formation of the tetrahedral intermediate are similar to those on the overall reaction.[12]

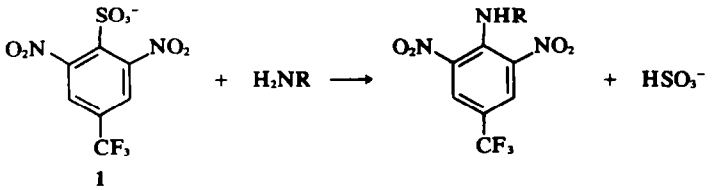
The overall reactions of the amines are shown below.

Benzene sulfonate ions, especially those containing *p*-alkyl substituents interact strongly with cationic micelles[13-15] which should readily incorporate 1. The substituents on the amino acids should also affect the interactions between the nucleophile and a cationic micelle, and we hoped to see some degree of specificity in these various reactions of 1 and DNF. This comparison should also illustrate the importance of charge type in micellar catalysis, because we have anionic or nonionic nucleophiles reacting with anionic or nonionic electrophiles.

RESULTS

Reactions in the absence of surfactant. For nucleophilic aromatic substitution by primary amino compounds upon DNF, addition is rate limiting,[11] although some reactions of secondary amines are general base catalyzed suggesting that breakdown of the tetrahedral intermediate then becomes slow.[16] Reactions of 2,4,6-trinitrobenzenesulfonate ion with primary amines are not base catalyzed at pH high enough for the amino group to be unprotonated,[10b] so that we can reasonably assume that nucleophilic addition is the rate limiting step for reactions of 1.

The amino acid anions are considerably more reactive towards 1 than is hydroxide ion (Table 1). This relatively low nucleophilicity of hydroxide could be caused by



1

R = $-CH_2CO_2^-$; $-CH_2CONH_2$; $-CH_2CONHCH_2CO_2^-$; $-CHPhCO_2^-$; $-CH(CH_2Ph)CO_2^-$; $-CH(CH_2CH(CH_3)_2)CO_2^-$

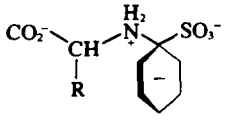
Table 1. Reactions of 1 and DNF with nucleophiles in the absence of surfactant[a]

Reagents	k_2, l. mole^{-1} sec^{-1}	
	1	DNF
glycinate	0·15	0·17[b]
glycylglycinate	0·026	0·034[b]
leucinate	0·029	0·099
phenylalaninate	0·013	0·117
phenylglycinate	0·061	0·128
glycineamide	0·037	0·019
OH^-	0·0013	0·12

[a]At 25·0°; [b]from Ref. 4.

unfavorable Coulombic interactions between the reacting anions.

The relative reactivities of 1 and DNF towards either glycinate or glycylglycinate are similar, which is expected because electron withdrawal by the trifluoromethyl group should compensate for the inherently lower reactivity towards nucleophiles of an arenesulfonate ion as compared with the corresponding fluoride, but there are marked differences in reactions with the other nucleophiles (Table 1). Steric effects of α-substitutents appear to be important in reactions of amino acid anions with 1, but not with DNF. The structure of the transition state should be similar to that of the intermediate,[11] and because of the negatively charged sulfonate group, conformation (3) should be preferred in reactions of 1, although it would bring bulky α-substituents close to the activated aryl group. This conformational preference should be less important in reactions of amino acids with DNF, which is uncharged, so that α-substituents should not sterically hinder the reaction, in agreement with the rate constants for reactions of the amino acid anions with DNF (Table 1).



3

Micellar effects

Reaction of 1 *with glycinate and glycylglycinate.* Reactions of the benzene sulfonate (1) with glycinate and glycylglycinate are modestly catalyzed by cationic micelles (Fig. 1), and the rate enhancements of approx. 6-fold by CTABr are much smaller than those found earlier for reaction with DNF.[4] In some aromatic nucleophilic substitutions, CTACl was a better catalyst than CTABr,[4] because bromide ions are better inhibitors than chloride ions of reactions catalyzed by cationic micelles.[6-9,17] Such an effect is absent here, probably because catalysis is observed with very low surfactant concentrations. The rate constants increase more gradually with CTACl than with CTABr. This chloride has a higher critical micelle concentration, cmc, than the bromide,[18] so that higher concentrations of surfactant will be required to take up the substrate fully. However rates are sharply enhanced below the cmc of the surfactants. This behavior is very common, especially when there are strong interactions between reagents and micelle.[6-9]

Added salts generally reduce micellar catalysis,[6-9,17] and this effect is observed here (Fig. 2). As expected for a

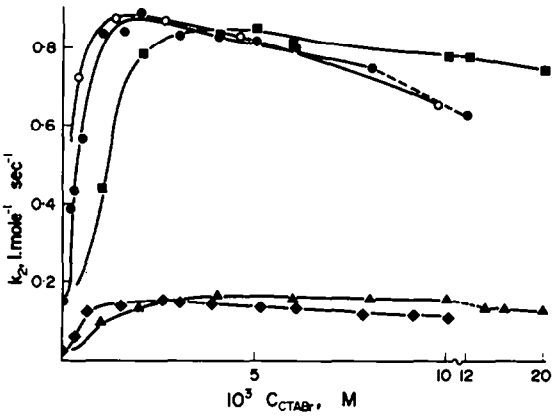


Fig. 1. Effect of cationic micelles upon the reactions of the arenesulfonate ion (1) with amines. Catalysis by CTABr: ●, 0·0255 M glycinate; ○, 0·0128 M glycinate; ◆, 0·0257 M glycylglycinate. Catalysis by CTACl: ■, 0·0255 M glycinate; ▲, 0·0257 M glycylglycinate.

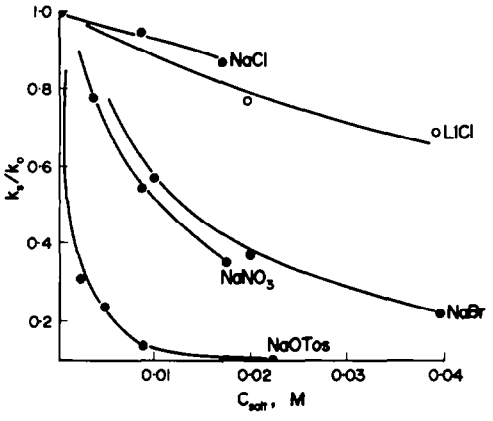


Fig. 2. Salt effects on the catalysis by 0·0019 M CTABr of the reaction of 0·0255 M glycinate with the arenesulfonate ion (1).

bimolecular reaction the inhibition increases with decreasing charge density (increasing hydrophobicity) of the salt anion, in the usual sequence: no salt $< Cl^- < Br^- < NO_3^- \ll OTos^-$. The relatively large salt effects are understandable, because anions of the added salt compete with both reagents for the cationic micelle.

Anionic micelles of NaLS do not inhibit the reactions of 1 with amino acids (Table 2) simply because both the anionic reagents are excluded from the micelles. The

Table 2. Effect of anionic micelles upon reactions of 1[a]

$10^3 C_{NaLS}$, M	Substrate	k_2, l. mole^{-1} sec^{-1}
	gly	0·150
4·0	gly	0·157
8·0	gly	0·163
16·0	gly	0·166
	glygly	0·026
4·8	glygly	0·020
9·6	glygly	0·020
14·4	glygly	0·020

[a]Reaction with 0·0255 M glycinate (gly) at pH 10·5 or 0·0257 M glycylglycinate (glygly) at pH 9·5.

corresponding reactions of DNF are strongly inhibited by anionic micelles.[2b,c,3,4]

Reaction of **1** *with anions of substituted amino acids.* Alkyl and aryl groups at the α-position retard reaction of amino acid anions with **1** in the absence of micelles (Table 1), but they increase the micellar catalysis (Fig. 3). The maximum rate enhancements due to CTABr are given in Table 3 together with the concentration of CTABr required for maximum rate enhancement. With phenylalaninate the micellar catalysis was relatively insensitive to the amino acid concentration, as shown by the second order rate constants (Fig. 3), although with the higher phenylalanine concentration the maximum value of k_2 is reached at higher CTABr concentration. This result is understandable in terms of the distributions of reagents between the aqueous and micellar phases.[19] (The rate constants were calculated from the total concentration of amino acid).

The reaction with glycineamide is unexpectedly not catalyzed by CTABr (Table 4).

Reaction of **1** *with hydroxide ion.* The reaction of hydroxide ion with DNF is effectively catalyzed by CTABr,[2b] but there is less catalysis of the reaction with **1** (Table 5) although in terms of Coulombic interactions, we expected this interanionic reaction to be effectively catalyzed by cationic micelles. In addition the micellar catalysis decreases very sharply with increasing hydroxide ion concentration. Possible reasons for the small catalysis are: (1) One anion may compete with the other

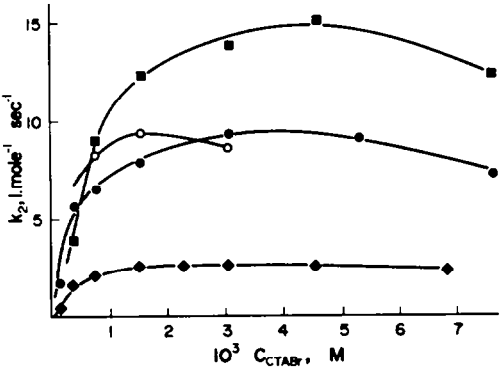


Fig. 3. Effect of CTABr upon reactions of the arenesulfonate ion (**1**) with: ●, 0·0128 M phenylalaninate; ○, 0·0064 M phenylalaninate; ■, 0·0123 M phenylglycinate; ◆, 0·0247 M leucinate.

Table 3. Effect of cationic micelles on reactions of **1**[a]

Reagent	k_{rel}	$10^3 C_{CTA}$(max)[b]
0·0128 M glycinate	5·8	2
0·0255 M glycinate	5·8	2
0·0255 M glycinate	5·7[c]	5[c]
0·0257 M glycylglycinate	6·0	2·5
0·0257 M glycylglycinate	5·4[c]	5[c]
0·0247 M leucinate	93	2
0·0128 M phenylalaninate	740	3
0·0064 M phenylalaninate	730	1·5
0·0123 M phenylglycinate	247	4·5
0·0252 M glycineamide	0·9	
0·0833 M NaOH	3	
0·167 M NaOH	1·6	
0·333 M NaOH	1	

[a]Rate constants relative to those in the absence of surfactant at 25° with CTABr unless specified; [b]surfactant concentration at rate maximum; [c]with CTACl.

Table 4. Reaction of **1** with glycineamide[a]

$10^3 C_{CTABr}$, M	k_2, l. mole^{-1} sec^{-1}
	0·037
0·38	0·031
0·76	0·032
1·52	0·032
3·04	0·030

[a]At pH 9·5 in 0·027 M Na_2CO_3 with 0·0252 M glycineamide.

Table 5. Reaction of **1** with hydroxide ion[a]

C_{NaOH}, M	$10^3 k_2$, l. mole^{-1} sec^{-1}
0·0833	3·70 (1·26)
0·167	2·94 (1·50)
0·333	1·63 (1·65)

[a]At 25·0° in $3·04 \times 10^{-3}$ M CTABr; the values in parentheses are in the absence of CTABr.

for the micelle, and increasing the hydroxide ion concentration tends to exclude **1** from the micelle, and salts decrease micellar catalysis of reactions with glycinate ion (Fig. 2). (2) The trifluoromethyl substituent makes **1** so hydrophobic that it is taken into a region of the micelle where the hydrophilic hydroxide ion does not penetrate.

Micellar effects upon reactions of DNF. Reactions of DNF with anionic and nonionic nucleophiles are catalyzed by cationic micelles (Fig. 4, Table 6 and Refs. 2–4), but the pattern is different from that for reactions of **1** in that for attack of amino acid anions the micellar rate enhancement is not especially dependent upon α-substituents in the amino acid, and the micellar catalysis is relatively large for reaction of the hydrophilic hydroxide ion (Tables 6 and Ref. 2*b*). The rate enhancement by CTABr which we observe for the reaction of glycineamide with DNF is small but similar to that reported earlier,[3] and contrasts with the absence of catalysis of the corresponding reaction of **1**. The smaller micellar catalyses of the reactions of DNF with glycineamide (or aniline[4]) relative to the amino acid

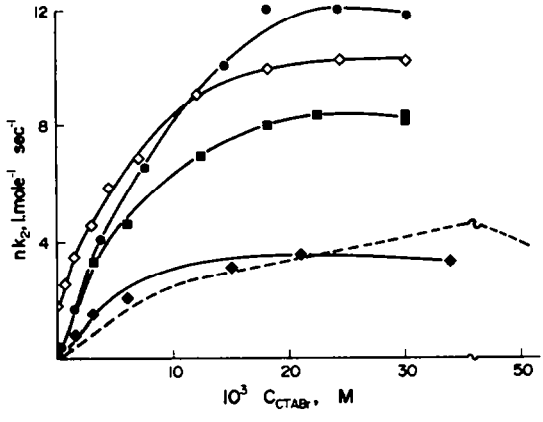


Fig. 4. Effect of CTABr upon reactions of DNF with: ● 0·0128 M phenylalaninate; ■ 0·0114 M phenylglycinate; ◆ 0·0246 M leucinate. ◇ 0·0308 M glycineamide. The broken line is for the reaction of glycinate, Ref. 4. n = 1 for reactions of the aminoacid anions and n = 100 for that of glycineamide.

Table 6. Effect of CTABr on reactions of DNF

Reagent	k_{rel}	$10^3 C_{CTA}(max)^c$
glycinate	28[a]	35
glycylglycinate	26[a]	25
leucinate	34	35
phenylalaninate	104	18
phenylglycinate	65	20
glycineamide	5·5	30
NaOH	60[b]	25

[a]Ref. 4; [b]Ref. 2b; [c]concentration at the rate maximum.

anions is understandable in terms of Coulombic interactions.

DISCUSSION

The relations between rate constant and concentrations of cationic surfactants are those expected for bimolecular reactions, with the rate constants increasing to maxima with increasing surfactant concentration.[6-9] These rate maxima have been explained in terms of either a negative electrolyte effect by the counterion of the surfactant[8] or a distribution of reagents between the micelles and bulk solvent.[2a,19] The simple quantitative treatment of micellar catalysis predicts no catalysis or inhibition below the cmc of the surfactant, but for these and many other reactions there are marked micellar effects at low surfactant concentrations, possibly because of catalysis by submicellar aggregates, but more likely because substrates, especially those which are hydrophobic, should reduce the cmc (*cf* refs 6–9, 18), and the sulfonate ion (1) should be particularly effective in this regard.[13-15]

Micellar catalysis of reactions of 1 *and DNF.* The micellar catalyses of reactions of nucleophiles with the sulfonate ion (1) or DNF are superficially similar, but there are marked differences in the overall rate enhancements by CTABr, which appear to depend upon the hydrophobicity of the nucleophile rather than on the charge type of the reaction (Tables 3 and 6).

Typically micellar catalysis increases with increasing reagent hydrophobicity[6-9] because drawing the reagent more deeply into the Stern layer of a micelle should increase the beneficial interactions between the micelle and the transition state.[20] This general pattern is followed in these aromatic substitutions on 1 because although the α-substituted amino acid anions are less reactive than the glycinate in the absence of surfactant (Table 1), probably because of steric hindrance by the α-substituents, the micellar catalysis is much greater (Table 3). The discrimination between reagents is much greater for reactions of the sulfonate ion 1 than of DNF (Table 6), and we suppose that these differences are related to the position of the reaction center in the Stern layer of the micelle. Arenesulfonate ions interact strongly with cationic micelles of tetraalkyl ammonium ions,[13] and consistently the reactions of 1 which are most strongly catalyzed are those with the more hydrophobic amino acids, with very little catalysis of the reactions with hydroxide ion or glycineamide (Table 5). On the other hand the reaction of hydroxide ion with DNF is strongly catalyzed by CTABr, and in general the catalyses of the reactions of the more hydrophilic nucleophiles, e.g. glycinate are much greater for DNF than for the sulfonate ion (1). These micellar catalyses therefore show considerable specificity towards the structure of the nucleophile, depending on the nature of aromatic substrate.

In considering the apparent steric hindrance by α-substituents to reactions of amino acids with 1, we noted the importance of unfavorable steric interactions in the intermediate (3), and in the transition state leading to it (Results). A transition state akin to 3 should however interact strongly with the surface of a cationic micelle, especially if the α-substituent, R, is, or contains, an aryl group, because aryl groups appear to insert into the micellar surface between the alkylammonium ion head groups.[13-15] Thus the structural features which reduce the reactivity of an amino acid anion towards 1 in the absence of micelles could assist the reaction in the presence of micelles.

Comparison of the pattern of micellar catalysis of the reactions of nucleophiles with DNF and 1 shows the limitations of treatments of micellar catalysis and inhibition based solely on Coulombic interactions between the reagents and the micelle. This approach is reasonably satisfactory for reactions of DNF, for example, micelles of CTABr effectively catalyze attack of various anions, and only weakly catalyze attack of uncharged nucleophiles, e.g. glycineamide[3] and aniline.[2a] But even here there are limitations to the explanation because reactions of hydrophobic anions, especially those having aryl substituents exhibit more micellar catalysis than those of hydrophilic anions. This approach fails completely for reactions of 1 where reactions of relatively hydrophobic nucleophiles, e.g. phenylalaninate and phenylglycinate ion are effectively catalyzed, but not those of hydrophilic nucleophiles, e.g. hydroxide and glycinate ions and glycinamide, irrespective of charge. There is extensive evidence that solutes can be incorporated at the micellar surface or in its interior, and effective catalysis requires the reactants to be in close proximity.

Reactions of both amines and anionic nucleophiles with halonitrobenzenes are faster in dipolar aprotic solvents than in water or alcohols, and it is reasonable to regard cationic micelles as providing a microenvironment for these reactions akin to a dipolar aprotic solvent (*cf* Refs 13 and 21).

Another striking difference between micellar catalyses of reactions of 1 and DNF is the concentration of CTABr required for maximum catalysis (Tables 3 and 6). The concentrations are much higher for reactions of DNF (20–35 mM) than for reactions of the arenesulfonate (1), where C_D (max) is 1·5–4 mM. These concentrations are not particularly dependent upon the nature of the nucleophile and they presumably indicate the relatively more favorable partitioning from water into the micelles of 1 as compared with DNF.

Both DNF and arenesulfonates such as 1 are useful protein modifying agents[10] and our observations suggest that it might be possible to discriminate between hydrophilic and hydrophobic regions of the protein by using both reagents.

EXPERIMENTAL

Materials. The purification of cetyltrimethylammonium chloride and bromide (CTACl and CTABr) and sodium lauryl sulfate (NaLS) followed standard methods.[2] Sodium 2,6 - dinitro - 4 - trifluoromethylbenzene sulfonate (1) was generously provided by Professors J. T. Gerig and J. Reinheimer.

The amino acids (Aldrich) were recrystallized (aq. EtOH) and dried at 80°, but the unrecrystallized and recrystallized materials gave the same rate constants. The L-enantiomers of the chiral amino acids were used.

Kinetics. The reactions of the amino acids in water at 25·0° were followed spectrophotometrically at 435 nm for **1** and 360 nm for DNF. The reaction of **1** with hydroxide ion was followed at 430 nm, using a Gilford spectrophotometer.[2]

All reactions were followed using a large excess of nucleophile over **1** or DNF which were 3×10^{-5} M. All reactions, except those with OH^- were made using 0·027 M carbonate buffer at a pH such that the amino group was not protonated, and the nucleophile was in the anionic form **2**.

$$RCH(NH_2)CO_2^-$$

Solns were made up in redistilled, deionized, CO_2 free water and freshly made up solutions of glycineamide were used to avoid hydrolysis.

The integrated rate constants, k_ψ, are in sec^{-1} at 25·0°, and second order rate constants, k_2, l. $mole^{-1}$ sec^{-1} were calculated by dividing k_ψ by the stoichiometric concentration of nucleophile.

Freshly prepared solutions of glycineamide were always used to avoid complications from hydrolysis.

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