Influence of cationic and anionic micelles on the reaction of ninhydrin with different amino acids: A kinetic study

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The kinetics of the reaction of ninhydrin with six amino acids in the presence of cationic micelles of cetyltrimethyl ammonium bromide (CTAB) and anionic micelles of sodium dodecylsulphate (SDS) have been studied spectrophotometrically at pH 5.0 at 575 nm. Both the micelles strongly inhibit the reaction due to the association of the substrate with the micelles. The results are best accounted for by the distribution of substrate into micellar and aqueous pseudo-phases as well as the combination of substrate molecules with surfactant molecules. From the kinetic data, micelle-substrate association constants ($K_s$), rate constant ($k_m$), fraction of substrate associated with micelles ($\alpha$), kinetic cmc value, index of cooperativity ($n$) and activation parameters have been calculated. The negative $k_m$ values suggest that the reaction does not occur in the micellar reaction zone (Stern-layer). The nonpolar side chain of amino acids play no significant kinetic role. Association of a dehydrated form of ninhydrin at the surface of the micelles is negligible.

Micelles are known to affect rates of reactions by partitioning the substrates between aqueous and micellar pseudophases and by purturbing the thermodynamic parameters of the reaction1. Micellar catalysis and inhibition have received considerable attention in view of the analogies drawn between the micellar and enzyme catalysis2. There are several examples of micellar effects upon the attack of nucleophilic anions on uncharged substrates3. Cationic micelles generally assist these reactions, and anionic micelles retard them as expected from electrostatic considerations and in some cases4 the reactive group has been incorporated into the surfactant structure. A scan through the existing literature reveals lack of work for micellar effect on molecule-molecule interaction whereas large number of data were found for micelle-ion-molecule reactions1,3.

The reaction between ninhydrin and amino acids has been studied extensively but largely in aqueous organic solvents5. However, the quantitative micellar effect has not been investigated on these reactions and we chose this reaction for initial study because the reaction proceeds through nucleophilic attack of the amino group on carbonyl group of ninhydrin. The substrates which were chosen for micellar-catalyzed reaction contain amino and carboxyl groups, both are responsible for completion of the reaction. The present work was undertaken to investigate the effect of cationic and anionic micelles on the reactivity of amino and carboxyl groups which are present at the same carbon. We examined the effects of substitution of different non-polar alkyl groups at the $\alpha$-carbon on the reaction rate. Propyl and methyl groups6 should have very similar electronic effects, but the propyl group should increase substrate hydrophobicity and might influence substrate orientation at the micellar surface and these studies are reported in this paper.

Materials and Methods

Glycine, alanine, valine, leucine, isoleucine, phenylalanine and ninhydrin were commercial samples (SRL, India). The ionic surfactants (Aldrich, India), cetyltrimethyl ammonium bromide ($C_{16}H_{33}NMe_3Br$, CTAB) and sodium dodecyl sulphate ($C_{12}H_{25}OSO_3Na$, SDS) were purified by repeated recrystallization. Deionized and redistilled, CO$_2$ free water was used to prepare solutions.

The cmc of the surfactants was determined by
the dye method using bromophenol blue for CTAB and methylene blue for SDS at 35°C with 0.05 mol dm⁻³ ninhydrin for CTAB, cmc = 0.92 × 10⁻³ mol dm⁻³ and for SDS, cmc = 8.0 × 10⁻³ mol dm⁻³. The cmc values are not particularly sensitive to small amounts of added amino acid.

**Kinetics**

The reaction between 0.05 mol dm⁻³ ninhydrin and 0.005 mol dm⁻³ amino acids in the presence of cationic and anionic micelles was followed spectrophotometrically at 575 nm using an Elico digital spectrophotometer. Surfactant was always added to the reaction vessel containing the required amount of amino acids. The reaction was initiated by mixing the required amount of ninhydrin and thermally equilibrated amino acid surfactant solution. Acetate buffer (0.02 mol dm⁻³) was used to control the pH. The first order rate constants with respect to amino acid were calculated by the conventional method and were designated as k_{obs} (s⁻¹). The results are reproducible within the limits of experimental error.

**Results and Discussion**

Preliminary studies showed the change in absorption maxima (λ_max) of the purple colour product of amino acid-ninhydrin reaction from 570 to 579 nm in the presence of CTAB micelles. However, no such change was detected in the presence of SDS micelles. This indicates ion-pair formation between the amino acid-ninhydrin purple product and the quaternary ammonium head group of CTAB in micelles.

The effects of varying concentrations of surfactants on the rates of amino acid-ninhydrin reactions were determined. The six amino acids with different alkyl side chains were used to investigate the effect of non-polar alkyl groups on the rate of reaction in the presence of cationic and anionic micelles. Pseudo-first order conditions were maintained in all kinetic runs using a large excess of ninhydrin (≥ ten fold). The results of a representative experiment are given in Fig. 1. The reactions were slower in CTAB than in SDS micelles at the same concentrations probably due to the strong association of CTAB micelles with substrate. It was observed that in CTAB, there is also inhibition below the cmc, therefore, all the kinetic studies were carried out above the cmc of CTAB.

In SDS the effect is large only above the cmc at which the surfactant abruptly associates to form micelles. The shapes of the curves of Fig. 1 indicate that simple 1:1 complexation between substrate (amino acid) and surfactant cannot be the cause of the rate inhibition. Cationic and anionic surfactants (CTAB and SDS) decrease the reactivity of amino acids towards ninhydrin.

Activation parameters (E_a and ΔH^* ) of the reaction have been calculated from the rate constants at 35, 38 and 45°C using Arrhenius and Eyring equations with the linear least squares technique. These values are summarized in Table 1 which indicate that the activation energy values continuously increase with increasing concentrations of CTAB and SDS. These results are consistent with the fact that slow reaction would require higher energy of activation. Values of rate constant (k_{obs}) and activation parameters for the six amino acids were found to be the same. Therefore, only one amino acid (alanine) was used for the calculation of other parameters. The values of E_a and ΔH^* in CTAB are greater as compared to the SDS which indicate the greater electrostatic attraction (strong binding) between the amino acid and CTAB micelles.

The pattern of micellar effects are quite different from those observed for other anion-molecule reactions where a cationic micelle assists the reaction, and an anionic micelle hinders it. The inhibition of the reaction between ninhydrin and amino acids by CTAB and SDS (Fig. 1) can be explained quite simply in terms of incorporation...
Table 1—CTAB and SDS dependent activation parameters for the alanine-ninhydrin reaction at 35°C

<table>
<thead>
<tr>
<th>10^3 [CTAB] (mol dm⁻³)</th>
<th>Eₐ (kJ mol⁻¹)</th>
<th>ΔH* (kJ mol⁻¹)</th>
<th>10^3 [SDS] (mol dm⁻³)</th>
<th>Eₐ (kJ mol⁻¹)</th>
<th>ΔH* (kJ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>19.13</td>
<td>16.58</td>
<td>0.00</td>
<td>19.12</td>
<td>16.57</td>
</tr>
<tr>
<td>1.0</td>
<td>24.12</td>
<td>21.56</td>
<td>10.17</td>
<td>25.00</td>
<td>22.45</td>
</tr>
<tr>
<td>5.0</td>
<td>38.00</td>
<td>35.44</td>
<td>12.72</td>
<td>27.80</td>
<td>25.25</td>
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<tr>
<td>8.0</td>
<td>49.21</td>
<td>46.65</td>
<td>15.90</td>
<td>29.44</td>
<td>26.89</td>
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<tr>
<td>10.0</td>
<td>57.41</td>
<td>54.86</td>
<td>19.00</td>
<td>31.12</td>
<td>28.57</td>
</tr>
<tr>
<td>15.0</td>
<td>70.00</td>
<td>67.55</td>
<td>22.21</td>
<td>32.10</td>
<td>29.55</td>
</tr>
<tr>
<td>20.0</td>
<td>78.12</td>
<td>75.56</td>
<td>25.00</td>
<td>34.81</td>
<td>32.26</td>
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<td>25.0</td>
<td>91.81</td>
<td>89.25</td>
<td>30.00</td>
<td>36.02</td>
<td>33.47</td>
</tr>
<tr>
<td>30.0</td>
<td>98.03</td>
<td>95.47</td>
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where \([S_a]\) = [Surfactant]_{total} - cmc, where cmc represents the critical micelle concentration and N is aggregation number. Equation (2a) is very useful in that it predicts that a plot of \(1/(k_w - k_{obs})\) against \(1/[S_a]\) should be linear and it allows the determination of both \(k_m\) and \(K/K/N\). The values of \(K/K/N\) and \(k_m\) are 1538.0, and \(-1.94 \times 10^{-6}\) s⁻¹ for CTAB and 74.1 and \(-1.96 \times 10^{-5}\) s⁻¹ for SDS respectively. The negative values of the rate constants of the reactions that occur in the micellar-pseudo phase suggest that no reaction occurs in this phase.

It is considered that the [amino acid] in the micelle increases with increase in [CTAB] and [SDS]. The amount of the amino acid present in the micelle \(\alpha\) can be calculated from its relative solubilities in water and surfactant solution using²ᵇ,⁹ Eq. (3).

\[
\frac{K_s}{N} = \left[ \frac{\alpha}{1-\alpha} \right] \left[ \frac{1}{(C_D - cmc)} \right] \tag{3}
\]

where \(C_D\) is the total surfactant concentration. The values of \(\alpha\) are given in Table 2. These values indicate that as [surfactant] increases in the reaction vessel, the amount of substrate in micellar pseudo-phase also increases and greater fraction of the substrate is solubilized in CTAB and SDS micelles leaving a smaller fraction in the aqueous phase. The solubilization of the substrate at apparently submicellar surfactant concentrations suggests that it is associating with the surfactant. A plot of \(\alpha/(1-\alpha)\) against \(C_D\) is linear.

The value of \(K_s/N\) calculated from the slope is 1550.0. The close agreement between the values of \(K_s/N\) (calculated from Eq. (1)≈ 1538.7) confirms the validity of Eqs (2a) and (3). The cmc va-
Table 2—Kinetically derived α and cmc in CTAB and SDS environments

<table>
<thead>
<tr>
<th>Temp.</th>
<th>10^4[CTAB]/mol dm^-3</th>
<th>10^4cmc/mol dm^-3</th>
<th>α %</th>
<th>10^4[SDS]/mol dm^-3</th>
<th>10^4cmc/mol dm^-3</th>
<th>α %</th>
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<tr>
<td>1.0</td>
<td>9.19</td>
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<td></td>
<td>10.17</td>
<td>8.13</td>
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<tr>
<td>5.0</td>
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<td>12.72</td>
<td>8.45</td>
<td>24.8</td>
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<td>9.22</td>
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<td>15.90</td>
<td>8.29</td>
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<td>19.00</td>
<td>8.33</td>
<td>44.9</td>
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<tr>
<td>15.0</td>
<td>9.46</td>
<td>95.6</td>
<td></td>
<td>22.20</td>
<td>8.10</td>
<td>51.3</td>
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<tr>
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<td>9.56</td>
<td>96.7</td>
<td></td>
<td>25.00</td>
<td>9.12</td>
<td>54.7</td>
</tr>
<tr>
<td>25.0</td>
<td>9.34</td>
<td>97.4</td>
<td></td>
<td>30.00</td>
<td>8.80</td>
<td>62.0</td>
</tr>
<tr>
<td>30.0</td>
<td>9.75</td>
<td>97.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Runs were duplicated.

Values have also been calculated from the kinetic data (Table 2). These cmc values are in the same order of magnitude, as determined experimentally.

The inhibition effect by CTAB below the cmc could be caused by the interaction between the substrate and submicellar aggregates of the surfactant which stabilize the initial state or the substrate might promote micellization of the surfactant by the formation of 1 : 1 molecular complex between the reagent and inhibitor.

The value of $K_s/N$ was found to be higher in CTAB(1538.70) inhibited reaction than in SDS (74.1). The higher $K_s/N$ could arise either from better substrate-micelle binding or from smaller aggregation number which would lead to more micelles for a given surfactant concentration or from the incorporation of more than one substrate molecule in a micelle. Herein we take the simplest assumption that a micelle accommodates only one substrate molecule. The aggregation number (N) for CTAB and SDS are 100 and 62, respectively, and therefore, $K_s = 153800.0$ and $4518.3$ respectively. The $K_s$ values indicate that there is strong substrate micelle binding in CTAB.

In a homogeneous surfactant solution (above cmc) the reactive site of a surfactant may exist in one or more of the following environments: the micelle interior (hydrophobic region), the micelle-water interface, hydrophilic region (Stern-layer) and the bulk solvent. NMR studies have shown that nonpolar aromatic compounds are absorbed into the interior of micelles, whereas polar aromatic compounds tend to stay in the exterior water rich region (Stern-layer). At pH 5.0, zwitter ionic form of amino acid is an active species. On the other hand, ninhydrin also participates in the hydrated and dehydrated form in the aqueous medium and the equilibrium states III and IV are to be considered.

Amino acids and ninhydrin are polar compounds and ready solubility of the ninhydrin in water depends upon the structure of (IV) which is more polar in comparison to (III). Out of the four species only I and II are active and responsible for the interaction of amino acid with ninhydrin. On the basis of this we are able to explain the inhibitory effect of CTAB and SDS on the rate of the reaction.

The remarkable rate inhibition and the negative value of rate constants ($k_m$) may be explained by amino acid adsorption within the outer aqueous areas of the micelles. The zwitter ionic form gets bound and forms the ion pair between the anionic site (COO^-) and the quaternary ammonium ion of the micellar head group of CTAB as well as the cationic site (-NH_3) with the carboxylate group of the micellar head of SDS. Therefore, both the micelles are very effective for incorporating II in the Stern layer. The equilibrium shifts towards the right hand side and the concentration of I decreases in the aqueous phase, retarding the reaction rate.

When the bulk of the amino acid is incorporated into the micelle, the addition of more surfactant provides more cationic and anionic micelles...
which simply take up I into the Stern layer, thereby deactivating it, because in the micellar reaction zone total substrate associates with the micelles in the form of II. As far as ninhydrin is concerned, the hydrated form (IV) may be present in the Stern layer. Therefore, in the Stern layer, both the reactants are present in the form of those species (viz. II and IV) which are unreactive towards the formation of imine bond (Schiff base). Out of these two forms (II and IV), II is also associated with the micelle. The foregoing discussion demonstrates that no reaction takes place in the micellar phase because a substrate in one micelle should not react with ninhydrin in another. Surfactants affect the properties of water itself and therefore should change the rate (and activation parameters) of that part of the reaction which occurs in the aqueous phase. In general, these effects appear to be less important than those caused by incorporation into the micellar phase.

To calculate the index of cooperativity \( [n] \) kinetic Scheme 2 was used. According to Scheme 2 a substrate molecule combines with \( n \) surfactant molecules to form a catalytic aggregate.

\[
\begin{align*}
&\text{S} + \text{nD} \xrightarrow{k_D} \text{Product} \\
&\text{S.D}_n^\text{n inhydrin} \xrightarrow{k_m} \text{ninhydrin} \\
&\text{Product}
\end{align*}
\]

Scheme 2

\[
\frac{k_{\text{obs}} - k_m}{k_m - k_{\text{obs}}} = n \log [C_D] - \log K_D
\]  

which predicts a linear relationship between \( \log ([k_{\text{obs}} - k_m]/(k_m - k_{\text{obs}})) \) and \( \log [C_D] \) with a slope equal to \( n \). In Eq. (4), \( [C_D] \) is the total surfactant concentration, \( K_D \) is the dissociation constant of surfactant-substrate complex and \( n \) is the index of cooperativity. Double log plot for the reaction in the presence of CTAB and SDS are linear. For the CTAB and SDS inhibited reactions, when \( k_m \) is assumed to be zero, the values of \( n \) obtained from the plot are 1.15 and 2.43 respectively.

The successful explanations of kinetic data based on both the kinetic Schemes to the reaction is not surprising. Though both the Schemes are based on different mathematical assumptions the final equation derived from them are similar (Eqs 2b and 4). The kinetic parameters associated with these equations have quite different significance. Equation (4) gives a set of parameters which have a different significance from that obtained from Menger and Portony’s Schemes, e.g., at \( \log \left( \frac{k_{\text{obs}} - k_m}{k_m - k_{\text{obs}}} \right) \mid m = k_{\text{obs}} = 0 \), \( n \log [C_D] = \log K \).

Scheme 2 draws its strength from a model used for the enzyme catalysed reactions showing positive homotropic interaction (called positive cooperativity). Positive cooperativity is reflected in the value of \( n \). In the micellar system, \( n \) gives the average number of surfactant molecules associated with each substrate molecule. Therefore, one molecule of CTAB and 2 molecules of SDS are associated with each molecule of alanine (substrate). On the other hand Scheme 1 is based on the distribution of substrate into micellar and aqueous pseudo-phases and has been extensively used to micelle catalysed and micelle inhibited reactions. The simultaneous use of both models provides a better insight and understanding of micellar effects on the reaction rate.

It was observed that alkyl chain has no significant kinetic role in the absence and presence of cationic and anionic micelles because the \( pK_1 \) and \( pK_2 \) values of all these amino acids are very close to each other. Methyl, propyl, and butyl groups should have similar electronic effects so comparison of the rate constants in the micellar pseudo-phases provides information on the effects of substrate location at the micelle-water interface (Stern layer). The rate constant \( (k_m) \) was found to be negative in this micellar reaction zone which indicates that no reaction takes place in this region. As the number of carbon atoms increase in the side chain it begins to incorporate into a micelle except phenyl group of phenylalanine which is more polar than alkyl group. Although phenyl groups enhance substrate binding, they do not affect the rate constant \( (k_m) \) for reaction in the micellar phase.

The primary amine head group \( (-\text{NH}_3^-) \) is more hydrophilic than alcohol, nitrile, carboxylate, ketone and aldehyde head group. Though the data are not conclusive, it seems that ninhydrin association with the micelles is negligible in comparison to amino acid. Therefore, the amino group interaction with middle carbonyl group of ninhydrin is not possible in the micellar reaction zone (Stern layer).

References


