Binding of Stearyltrimethylammonium Chloride to Human Hair

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The binding isotherms of a cationic surfactant of a long alkyl chain, stearyltrimethylammonium chloride, to three types of human hair (unaltered, bleached, and permanent-waved) were obtained and analyzed. The isotherms showed a Langmuir-type binding, from which the binding constant, the number of binding sites in saturation, and thermodynamic parameters were calculated. The binding mechanism is discussed from the point of view of hydrophobic and electrostatic interactions, and the location of binding sites estimated by a surface analysis of the hairs by X-ray photoelectron spectroscopy (XPS).

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INTRODUCTION

Hair is an important component of the human body. It serves the role of protection from physical trauma, extreme temperature change, and electromagnetic radiation as well as being a means of adornment and communication. Scientifically, many studies have been done from the point of view of the biochemical and biophysical behaviors of human hair (Robbins 1994) as well as its molecular and structural biology (Kurt et al. 1991).

In studies on human hair, the interaction of hair with chemical substances, such as cosmetics, dyes, shampoos or conditioners is a major subject. In particular, the binding of surfactants to human hair is interesting and significant because in daily life hair and skin are frequently exposed to various surfactants. Shampoo and hair conditioner are typical examples. The major component of hair conditioner is a cationic surfactant with a long alkyl chain such as stearyltrimethylammonium chloride, cetyltrimethylammonium chloride, or dioctadecyldimethylammonium chloride. These surfactants are also an ingredient in conditioning shampoo. The binding behavior of these surfactants should be elucidated in order to obtain insight into the mechanism of the action of surfactants on human hair.

Binding of ionic surfactants to water-soluble proteins has been widely studied (Steinhardt and Reynolds 1969; Nozaki et al. 1974). In these investigations it was clarified by analyzing the binding isotherms in connection with the conformational change in proteins that the binding is derived by both hydrophobic and ionic interactions. Binding of ionic surfactants to human hair has also been studied (Ohbu et al. 1986). However, the surfactants examined in these studies were mostly those with alkyl chains of less than twelve carbon atoms. Surfactants with a longer alkyl chain should also be investigated in detail.

In this paper, the binding behavior of stearyltrimethylammonium chloride (STAC) to human hair was investigated by analyzing binding isotherms of STAC to three types of hair (unaltered, bleached, and permanent-waved) and by analyzing the hair surface mainly by X-ray photoelectron spectroscopy (XPS). The binding scheme of STAC to human hair is then discussed.

MATERIALS AND METHODS

Chemicals

All chemicals used are commercially available. STAC was purchased from Tokyo Kasei Co. and used after being recrystallized six times with acetone.

Hair samples

Hair samples (unaltered, bleached, and permanent-waved hair) were prepared according to the literature (Ohbu et al. 1986). The unaltered hair sample was prepared as follows. Human hairs from males aged 20-22 years who had not bleached, dyed, or permanent-waved their own hair for 3 years prior to cutting, were cut and collected. The hairs were gently
washed with a 1 % polyoxyethylene lauryl ether (degree of polymerization, 10) solution, followed by rinsing with deionized water for 5 h, with ethyl ether for 5 min, with ethyl alcohol for 5 min, and with distilled water for 48 h successively, then dried in a desiccator over P2O5.

Damaged hair samples were prepared from the unaltered sample. To obtain a bleached hair sample, unaltered hair was immersed in 6% hydrogen peroxide solution for 1 h, rinsed with distilled water for 5 h and dried. A permanent-waved hair sample was obtained by immersing unaltered hair in 5% ammonium thioglycolate solution for 30 min, followed by dipping into ammonium hydroxide solution (pH 9.3) for 30 min. The sample was then rinsed with distilled water for 24 h and dried.

**Determination of bound surfactant**

A hair sample (0.2 g) was placed in a flask containing 20 ml of STAC solution of various concentrations. Phosphate buffer (0.067 M, pH 5.2) was used unless otherwise noted. The flask was shaken in a thermostat for 24 h. After removing the hair, the STAC concentration of the solution was determined by the methylene blue method (Ka-rikome 1975) or tetraphenylborate method (Uno et al. 1960). The amount of bound surfactant was calculated from the decrease in STAC concentration upon binding to hairs in comparison with the blank.

**Instrumental analysis**

XPS spectra were recorded on a Shimadzu ESCA-850 equipped with an AlKα radiation source at 1,487 eV and 300 W power at the anode. Nitrogen 1p-core level and sulfur is-core level spectra were taken to analyze the hair surface as well as wide spectra. FT-IR spectra were taken on a Perkin-Elmer System 2000 by ATR method. Scanning electron microscopy (SEM) was done on a Hitachi H-8100 after treating a hair sample with Pt-Pd ion-sputtering. Relative surface area was measured on a Shimadzu Micrometrics ASAP-2010M by BET method using nitrogen adsorption.

**RESULTS AND DISCUSSION**

**Binding isotherms and binding characteristics**

Figure 1 shows the binding isotherms of STAC to the weight g of hair to the three hairs at 30 and 40°C. In all cases the amount of bound STAC increased continuously with the equilibrium concentration of the surfactant and reached a constant value, indicating a Langmuir-type binding. The amount of STAC bound to permanent-waved hair was larger than that bound to unaltered or bleached hair. The amount was larger at 40°C than 30°C for the three types of hair.

Langmuir-type binding can be described by a simple equation (Eq. (1)) where \( r, n, K \) and \( C \) are the amount of bound surfactant, the amount of binding sites in saturation, a binding constant and the equilibrium concentration of surfactant, respectively.

\[
\frac{1}{r} = \frac{1}{(1/C)} + \frac{1}{nK} + \frac{1}{n} 
\]

(1)

The values of \( n \) and \( K \) are obtained from the plot of \( 1/r \) versus \( 1/C \). Figure 2 shows the reciprocal plots. The intercept and slope with a linear relationship gave the values of \( n \) and \( K \) for the STAC binding to solutions with low STAC concentration of less than 0.75 mm, because for the solutions of higher concentration it was sometimes hard to separate the aqueous layer and the chloroform layer due to the increased miscibility of the two layers in the presence of a large amount of STAC and SDS. The tetraphenylborate method was applicable to solutions with high STAC concentration of more than 0.50 mm, because for solutions of less STAC it was sometimes hard to detect a color change with the indicator.
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The tendency of binding of surfactant to hair is determined mainly by two factors: number of binding sites and strength of the binding. The former and the latter are estimated by \( n \) and \( K \) respectively in the case of Langmuir-type binding. The amount of bound STAC to permanent-waved hair was larger than those to the other two types of hair as shown in Fig. 1. From Table 1 it is seen that the increase in bound STAC in permanent-waved hair was brought about by the increase in both \( n \) and \( K \). The value of \( n \) of permanent-waved hair was almost twice as large as that of unaltered or bleached hair. The value of \( K \) was larger than that of unaltered hair, but comparable to that of bleached hair.

Another characteristic of the isotherms is that no cooperative binding is seen in the STAC-hair system. Cooperative binding has often been reported in the binding of ionic surfactants to water-soluble proteins, which accompanies a conformational change in proteins that exposes hydrophobic regions to an aqueous phase (Steinhardt and Reynolds 1969; Nozaki et al. 1974). The binding consists of two stages in case of water-soluble proteins; at lower surfactant concentrations below cmc the binding shows no cooperative increase followed by a sudden increase in the binding isotherms at higher surfactant concentrations. Each stage shows a Langmuir-type binding. In the first stage each surfactant molecule

![Fig. 1. Binding isotherms of STAC to unaltered hair (---), bleached hair (--), and permanent-waved hair (--Δ--) at 30°C (A) and 40°C (B)](image)

![Fig. 2. Reciprocal plots for the binding isotherms of STAC to unaltered hair (---), bleached hair (--), and permanent-waved hair (--Δ--) at 30°C (A) and 40°C (B)](image)
binds to an independent site. In the second stage, micell-like clusters are successively formed surrounding the previously occupied binding sites of the protein.

Cooperative binding was also observed in the binding of SDS to human hair, especially to damaged hairs (Ohbu et al. 1986). However, in the binding of a cationic surfactant, decyltrimethylammonium chloride (DTAC), to human hair no cooperative binding was seen (Ohbu et al. 1986). It is then expected to make it clear if no cooperative binding occurs in the binding of a cationic surfactant of a longer alkyl chain.

In the present study of the STAC-hair system, no cooperative binding was observed either even at STAC concentrations far above its cmc value, which is 0.34 mM at 35°C (Nakayama and Yamaguchi 1992). Cooperative binding accompanies structural changes in protein molecules which include the backbone. However, in the STAC-hair system a large conformational change in fibrous keratin is unlikely. FT-IR spectra of the three hairs demonstrated that no structural change in the main chain of keratin fiber occurred because both amide I (1,635 cm⁻¹) and amide II (1,519 cm⁻¹) bands of the three hairs were the same as those of STAC-bound hairs. XPS spectra of sulfur in hair indicated cleavage of disulfide linkages in bleached hair, but no cleavage in permanent-waved hair, as will be shown later. The cleavage of S-S bonds might partly contribute to the conformational change in side chains but not to a structural change in the protein backbone.

In conclusion, each STAC molecule binds to a binding site in keratin fiber independently and no further binding around the bound STAC occurs, probably because of the lack of a large conformational change in the keratin backbone.

Table 1. Binding constant (K), amount of binding sites in saturation (n), and thermodynamic parameters for the binding of STAC to human hair

<table>
<thead>
<tr>
<th>Hair</th>
<th>Temp (°C)</th>
<th>n (10⁻⁴ mol/g)</th>
<th>K (10⁻⁴/lmol)</th>
<th>−ΔG (kJ/mol)</th>
<th>−ΔH (kJ/mol)</th>
<th>−ΔS (J/mol-deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaltered</td>
<td>30</td>
<td>23.0±1.0</td>
<td>3.5±0.3</td>
<td>20.6</td>
<td>0</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>35.9±3.9</td>
<td>2.6±0.6</td>
<td>20.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleached</td>
<td>30</td>
<td>25.0±2.5</td>
<td>9.0±1.3</td>
<td>22.9</td>
<td>310</td>
<td>1,100</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>33.6±3.7</td>
<td>21.9±2.4</td>
<td>38.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent-waved</td>
<td>30</td>
<td>43.0±4.3</td>
<td>8.9±0.9</td>
<td>22.7</td>
<td>446</td>
<td>1,550</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>60.3±4.0</td>
<td>18.3±2.6</td>
<td>33.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures 3 and 4 show the pH dependence and ionic strength of the binding. As pH rises, bound STAC increases, suggesting that the binding comes from ionic interactions between the positively charged ammonium group of the surfactant and anionic groups of the protein side chains. As the isoelectric point of human hair is reported to be 3.67 (Wilkerson 1935), human hair would possess a net negative charge in the solution under the same condition of pH 5.2 as used here. An ionic interaction could then contribute to the association.

Figure 4 shows the effect of ionic strength of the solution on the binding. The amount of bound STAC increased with the ionic strength of the solution. This relationship suggests that a hydrophobic interaction contributes to the binding because a large number of ions in the solution work favorably for the association of the hydrophobic portion of molecules, such as the stearyl group of STAC and the hydrophobic side chains of keratin, known as a salting-out effect.
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(Timascheff and Fasman 1969).

In the following, the nature of the forces for the binding is discussed in more detail together with the thermodynamic parameters of the binding. In Table 1 are listed the thermodynamic parameters of the binding. A free energy change, $-\Delta G$, is obtained from the binding constant, $K$, using the following equation (Eq. (2)).

$$-\Delta G = R \cdot T \cdot \ln K$$

The enthalpy change ($\Delta H$) and entropy change ($\Delta S$) of the binding are obtained from the free energy changes at two different temperatures using the following equation (Eq. (3)).

$$-\Delta G = -\Delta H + T \cdot \Delta S$$

The large $K$ values, and hence large $-\Delta G$ values, of the damaged hairs shown in the table might result partly from the formation of a sulfonic group by the cleavage of the disulfide bond of the keratin fiber. The cleavage can be detected by XPS spectra of sulfur in keratin. A wide spectrum of the STAC-bound hair shows the atoms on the keratin fiber, as in Fig. 5. Figure 6 shows the S 2p XPS spectra of the three hairs. In the spectra of bleached hair a small peak appears at 168.7 eV besides a peak at 164.4 eV. The former peak corresponds to $-\text{SO}_3^-$ linkage formed by the scission of disulfide bonds, and the latter to disulfide bond of keratin (Robbins and Bahe 1984). On the other hand, no cleavage of disulfide bonds was seen in permanent-waved hair, because the S 2p spectrum of the hair was almost the same as that of unaltered hair. Formation of sulfonate by oxidation of S-S bond with hydrogen peroxide would contribute to

the increase of $-\Delta H$. However, despite the formation of the anionic group, the enthalpy change is more endothermic in the binding of bleached hair than unaltered hair as seen in Table 1. The enthalpy change in permanent-waved hair is also endothermic, in which no formation of sulfonate groups is detected, although the reason for the endothermic enthalpy change in damaged hair is unclear.

A large entropy change is indicated in the binding of the two types of damaged hair instead, which cancelled the unfavorable $-\Delta H$. A large entropy change has also been reported in the binding of ionic surfactants to soluble proteins. It was explained that a hydrophobic interaction is predominant in the binding, because hydrophobic interactions usually accompany a large entropy change (Tanford 1979). If that
is true, in the present investigation hydrophobic interactions would also be predominant in the binding of the damaged hairs, which would accompany the disorder of protein conformation, probably in the side chain conformation, and water structure.

To summarize, electrostatic interactions contribute only slightly to $-\Delta G$. The binding is dominated mainly by entropy changes which might be the result of hydrophobic interactions.

**Location of binding site**

The binding scheme of STAC to the three hairs is considered in connection with the location of binding sites. SEM pictures of the hairs are shown in Fig. 7. It is seen in the figure that permanent-waved hair is highly damaged compared to the other two types of hair. SEM pictures of STAC-bond hairs were almost the same as those of unbound hairs. Morphologically, it might be said that the increase in the binding sites, as indicated by the large $n$ values of permanent-waved hair, could be accounted for by the damaged surface of permanent-waved hair.

However, XPS spectra offer a strong argument against the above idea. XPS is used to identify atoms and analyze their nature, or the chemical bonds located at or near the surface of various materials, by observing the energy of photoelectrons (binding energy) emitted from an atom through the X-ray radiation. The position of the peak, that is, the value of the binding energy, provides information on the chemical composition of substances on the surface of the material and on the state of the chemical bonds. Peak area gives the relative amount of an atom. Information gained by XPS is relevant to atoms located to the depth of 3-10 nm in polymer materials (Kobunshi Gakkai 1996).

Figures 8 and 9 show the N 1s spectra of hairs. In the spectra of the surfactant-bound hair there are two peaks at 400.6 eV and 403.1 eV. The peak appearing at 403.1 eV is attributed to the ammonium nitrogen in STAC and the one at 400.6 eV to the nitrogen in keratin fiber. It is clearly demonstrated from the spectra that STAC does bind to the hair surface. The ratio of the areas under the two peaks arising from nitrogen in hair and bound STAC is 1 : 0.22, 1 : 0.21, and 1 : 0.20 for unaltered hair, permanent-waved hair, and bleached hair, respectively. The ratio indicates the relative amount of STAC and amino acid residues in keratin on the hair surface. An interesting point is that there is no significant difference in the relative areas of the two peaks of STAC-bound permanent-waved hair, bleached hair and unaltered hair.
although the amount of bound STAC to permanent-waved hair is twice as large as the other hairs. This means that maximum binding at the surface occurred and the number of binding site of the three hairs is almost the same despite the differences in the three hairs. The ratio of the two peak areas makes it possible to evaluate the maximum binding number on the hair surface; that is, one STAC molecule per 5 amino acid residues of keratin regardless of the nitrogen of the side chains in keratin.

The difference in the number of binding sites \( n \) among the three hair types should then be explained in some way other than the binding of STAC at the hair surface. The above data suggest that the increase in binding sites in permanent-waved hair comes from the increase in available binding sites far beneath the hair surface to which the X-rays of XPS cannot reach. Moreover, the relative surface area of the three hairs did not differ greatly, being ca. 0.5 by the BET method. The resemblance of the surface area of the unaltered hair and the two damaged hairs also supports the above scheme.

The binding of chemical substances to human hair consists of two processes: an adsorption to human hair and diffusion into human hair. The XPS data strongly suggest that the increased binding of permanent-waved hair resulted from the increased adsorption to and/or diffusion into the portion of hair which surfactants usually cannot enter. For such diffusion two pathways exist: (1) transcellular diffusion and (2) intercellular diffusion. The transcellular diffusion involves diffusion across cuticle cells through highly cross-linked proteins. On the other hand, intercellular diffusion involves penetration between cuticle cells through low cross-link density regions of the intercellular cement. Today it is believed that the intercellular diffusion through the nonkeratinous regions of the intercellular cement is the preferred route of entry of most molecules, especially large ones such as surfactants (Robbins 1994).

The results here then support the scheme for the increased binding in which the permanent-wave treatment lifted or loosened the cuticle cells which cover the cortex of human hair, as shown in Fig. 10, enabling more surfactants to adsorb to or diffuse into inner nonkeratinous regions, where STAC cannot reach in the case of unaltered or bleached hair.

Greatful acknowledgement is made to Shimadzu Seisakusho Co. for measuring the relative surface area.

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Fig. 8. N 1s spectra of unaltered hair (a), bleached hair (b), permanent-waved hair (c), STAC-bound unaltered hair (d), STAC-bound bleached hair (e), STAC-bound permanent-waved hair (f), and STAC (g) (200 scans)

Fig. 9. Comparison of N 1s spectra of STAC-bound unaltered hair (a) and STAC-bound permanent-waved hair (b) (dotted lines designate the Gaussian approximation of the spectra)
毛髪への塩化ステアリルトリメチルアンモニウムの結合

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長鎖イオン界面活性剤，塩化ステアリルトリメチルアンモニウムの，3種類の毛髪（未処理，漂白処理，コールドパーカーマ処理）への結合等温線を求め，それを解析した．結合等温線は，Langmuir型であり，その解析から，結合定数，飽和結合率，結合の熱力学的パラメータが計算された．結合機構を，疎水性相互作用，静電相互作用の観点から議論した．そして，主として，X線光電子分光分析（XPS）を用いた毛髪の表面分析から，結合場所を推定した．

キーワード：毛髪，塩化ステアリルトリメチルアンモニウム，結合等温線，Langmuir型結合，XPS,