The Interaction of Bovine Plasma Albumin with Cationic Detergent. Studies by Binding Isotherm, Optical Rotation and Difference Spectrum

Koichi HIRAMATSU, Chiaki UEDA, Koji IWATA, Kinichi ARIKAWA, and Koichiro AOKI

Department of Synthetic Chemistry, Faculty of Engineering, Gifu University, Kagamigahara, Gifu 504

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Binding isotherms were determined at pH 6.9 for systems of bovine plasma albumin (BPA) and cationic detergents at 25° C and 5 °C. Detergents used were: hexadecyltrimethylammonium bromide (HTAB), tetradecyltrimethylammonium bromide (TTAB), dodecyltrimethylammonium bromide (DTAB) and decyltrimethylammonium bromide (DeTAB). Binding affinity of the cationic detergent to BPA increased with the increase in the carbon number of the detergent, and with the increase in temperature. The first five detergent ions were bound to BPA statistically at 25 °C, and succeeding detergent ions were bound cooperatively. Thermodynamic parameters indicated that the statistical binding was caused mainly by the hydrophobic bonding. Measurements of $-[\alpha]_{233}$ and $-[\alpha]_{313}$ at pH 5.2 revealed that the conformation of BPA changed when it complexed with the cationic detergent. The conformation of BPA changed slightly when 5—8 HTAB or TTAB's were bound, and a second large conformational change occurred when 15—20 of these detergent ions were bound. DTAB and DeTAB caused only the first conformational change. Thus HTAB and TTAB are stronger unfolders of BPA than DTAB and DeTAB. The UV difference spectrum of the complex BPA–TTAB showed a red shift of the peak of Try residue (*e. g.* 292 nm), being in contrast to the blue shift of the same peak in the complex BPA–sodium dodecyl sulfate. It is suggested that BPA is unfolded, at least, in the NH_2 terminal half by binding with cationic detergent.

In a previous paper,[1] it was reported that the binding of cationic detergent to BPA (bovine plasma albumin) at pH 9 induced the SH–S-S exchange reaction of BPA, forming a series of aggregates of BPA. The exchange reaction occurred at lower concentration of the detergent, when the carbon number of detergent was higher. It was suggested that the first step of the reaction is the unfolding of BPA by the cationic detergent.

This time studies were made on the binding behavior of cationic detergents to BPA and on the conformational change of BPA caused by the cationic detergent. Studies were made by equilibrium dialysis, by measuring the optical rotations at 233 and 313 nm, and by measuring the UV difference spectrum. Some aspects of the unfolding of BPA by the cationic detergent were made clear at pH 5.2 and 6.9. Cationic detergents used were:
hexadecyltrimethylammonium bromide (HTAB),
tetradecyltrimethylammonium bromide (TTAB),
dodecyltrimethylammonium bromide (DTAB), and
decyltrimethylammonium bromide (DeTAB).

So far, several works have been made on the interaction between BPA and cationic detergents.[1-5]

Experimental

Materials. Crystallized BPA (Armour, Lot No. M-72603) was used. A part of the experiment was performed using BPA whose SH group was blocked by iodoacetamide. The blocking was made at room temperature and pH 8.0 (in 0.1M KCl+KOH) for 3 h. Then the solution was dialyzed against the deionized water at 5 °C for more than 24 h or passed through the Sephadex G-25 gel (Pharmacia Fine Chemicals, Lot No. 0063) column. Frequently, contaminants were removed by filtering the solution through Millipore filter (HATF, 02500). The concentration of BPA was determined spectrophotometrically using the value $E^{1\%}_{1cm}=6.67$ at 279 nm.

Buffers used were: phosphate buffer at pH 6.9 and ionic strength 0.1, and $Na_2B_4O_7$–HCl buffer at pH 9.0 and ionic strength 0.025 (sometimes the ionic strength was raised to 0.1 by adding KCl). BPA was also dissolved in 0.1 M KCl; the isoionic point was pH 5.2.

Cationic detergents used were the same samples as those described in a previous paper.[1]

Equilibrium Dialysis. The Visking tube (20/30) was heated in a half saturated solution of $NaHCO_3$ at 90 °C for 1 h, then the tubings were rinsed well with deionized water. Five ml of 0.5% BPA was dialyzed against 25 ml of detergent solution. The dialysis was conducted for 65 h at 5 and 25 °C.

Determination of the Cationic Detergent. The determination was carried out following the method of Few and Ottewill.[6] The method involves the procedure to extract the detergent–Orange II complex in the aqueous phase by the organic solvent. In the present work, the extraction solvent was dichloromethane instead of chloroform. The optimum conditions for the extraction were: concentration of the complex is to be in the range 9×10^{-6} and 3×10^{-5} mol/l, and the mol ratio of Orange II to detergent is to be in the range 1—3. When the concentration of the detergent in the sample solution was higher than the optimum value, the solution was diluted by the same buffer as was used for the sample solution. The extinction coefficient of the complex at 485 nm was 1.80×10^4 mol^{-1} cm^{-1} in presence or absence of ethanol (antifoaming agent), and was 2.20×10^4 mol^{-1} cm^{-1} in aqueous phase. The extinction coefficient of the complex in distilled water was equal to that of Orange II.

Optical Rotation. The optical rotation was measured using 0.2% BPA at various concentrations of detergent. The 1 mm cell and 10 mm cell were used to measure the optical activity at 233 and 313 nm, respectively. To increase the sensitivity of the measurements, compensation by means of sucrose was employed.[7] The temperature was kept constant at 25±0.2 °C. The apparatus was a Jasco spectropolarimeter, model ORD/CD/UV-5.

Difference Spectrum. The difference spectrum was measured using 0.15% BPA in presence of various amounts of detergent. Using 10 mm cell, the spectrum was recorded in the range 275—350 nm. The temperature of the cell was kept constant at 25±0.2 °C. The apparatus was a Hitachi EPS-3 spectrophotometer.

Results and Discussion

Equilibrium Dialysis. *a) Binding Isotherms at pH 6.9*: In Fig. 1 are shown binding isotherms of the systems of BPA and each one of the detergents, HTAB, TTAB, DTAB, or DeTAB at pH 6.9 and 25 °C. All of the curves consist of two parts: one of which lies in the lower concentration of the detergent and has a small slope and the other is the curve having a large slope. It is known later that the binding of the detergent to BPA is statistical in the lower concentration region of the detergent, and that it is cooperative in the higher concentration region. The whole binding isotherm shifts to the left with the increase in the carbon number of the detergent. In other words, the binding occurs at lower concentrations of detergent, when the detergent with higher carbon number is used. This suggests that the hydrophobic group of the detergent is more effective than the charged group for the BPA–cationic detergent interaction.

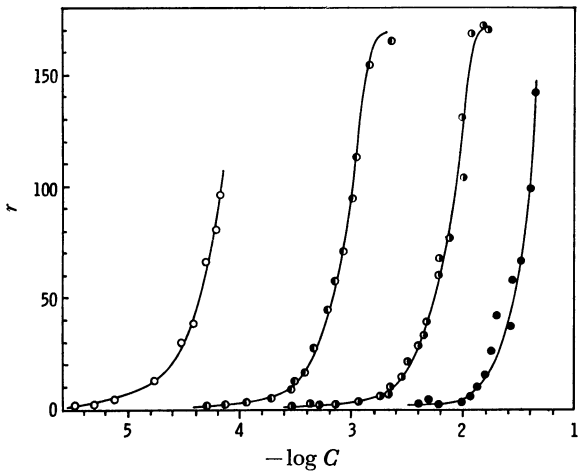

Fig. 1. Binding isotherms of the systems of BPA and cationic detergents at pH 6.9, ionic strength 0.1 and 25 °C. *C*: equilibrium concentration of detergent (mol/l), *r*: number of detergents bound to BPA. ○: HTAB-BPA, ◐: TTAB-BPA, ◑: DTAB-BPA, ●: DeTAB-BPA.

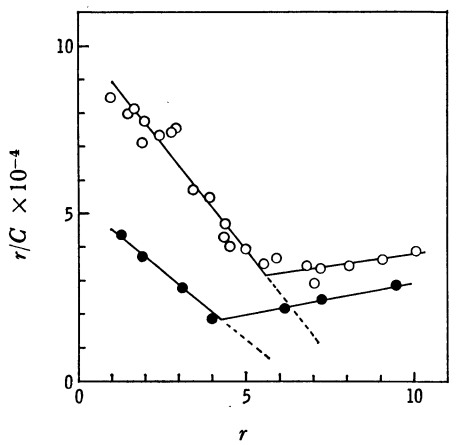

Fig. 2. Scatchard plot for the system BPA-TTAB. ○: 25 °C, ●: 5 °C.

When the temperature is increased, the binding isotherm shifts to the left, indicating that the binding is increased with the increase in temperature, *i.e.*, the binding is endothermic. The result agrees with that by calorimetric measurement by Jones *et al.*[5] The effect of temperature is shown in Fig. 2.

The binding isotherm was analyzed by the Scatchard equation.[8]

$$r/C = k_0 n - k_0 r \quad (1)$$

where, C: equilibrium concentration of detergent, n: maximum number of the binding sites, r: average number of the detergent ions bound and k_0: intrinsic binding constant. The application of the equation to the system BPA–TTAB, as an example, is given in Fig. 2. There are two straight lines with a break. The one is that for 25 °C, and the other is that for 5 °C. The straight line with a negative slope satisfies the Eq. 1, meaning that the binding is statistical. The straight line with a positive slope gives a negative value of k_0; the meaning is that the binding is cooperative.

Figure 2 shows that $r_{s,max}$ is 5.5 at 25 °C.* If the straight line with a negative slope is extended, it is found that the maximum number of sites n should be 8. The value of $r_{s,max}$ is 4 at 5 °C. Values of $r_{s,max}$ for other detergents are given in Table 1. It was difficult to find accurate values for DeTAB. This might be due to the difficulty in analytical procedure.

b) Statistical Binding: As is seen in Table 1, $r_{s,max}$ does not depend on the carbon number of the detergent, *i.e.*, it is 4 at 5 °C and 5 at 25 °C. The value agrees with the value 4 by Nozaki *et al.*[4] for tetradecyltrimethylammonium chloride at pH 5.6 and 24 °C within the experimental error, and agrees also with the value 6 by Few *et al.*[2] at the isoionic point and 20 °C for DTAB. When 4 or 5 cationic detergent ions are bound to BPA, slight unfolding occurs in BPA as is described later, thus $r_{s,max}$ is constant, *i. e.*, more binding sites with high affinity are destroyed when unfolding occurs. If the unfolding does not occur before all the n sites are occupied, $r_{s,max}$ would be equal to n.

The value of $r_{s,max}$ is 4—5 for the cationic detergents with C_{12} and C_{14}, but it is 8—10 for the anionic detergents with C_{12} and C_{14}.[9,10] (Decker and Foster[9] reported that $r_{s,max} \fallingdotseq n = 10$ for sodium dodecylbenzenesulfonate.) Such a difference in numericals exists between the cationic and anionic detergent bindings. It is well known that the BPA unfolds when the number of anionic detergents bound exceeds the value of $r_{s,max}$.[10]

c) Thermodynamic Properties of Statistical Binding: In Table 1 are given values of k_0 and thermodynamic parameters $-\Delta F$, ΔS, and ΔH for the interactions. It is seen that $H>0$ and $\Delta S>0$, and that $T\Delta S>\Delta H$. This indicates that the binding of the cationic detergent

* The notation $r_{s,max}$ means the maximum number of the detergent ions bound statistically. As is known from Eq. 1, the crossing point of the straight line with a negative slope and the abscissa gives the value of n. The break of the straight line in Fig. 2 is named $r_{s,max}$ to distinguish it from n.

Table 1. Thermodynamic parameters for BPA-cationic detergent interactions

Detergent	Temp °C	$r_{s,max}$	k_0 l/mol	$-\Delta F$ kcal/mol	ΔH kcal/mol	ΔS e. u.
HTAB	25	5	8.7×10^4	6.7	4.0	36
	5	4	5.2×10^4	5.8		
TTAB	25	5.5	1.3×10^4	5.5	3.5	30
	5	4	8.2×10^3	5.0		
DTAB	25	5	9.7×10^2	3.9		

to BPA is entropic. This means that the interaction between the detergent and the solvent molecule is replaced by the interaction between the detergent and BPA, *i. e.*, that hydrophobic bonds are formed. It is known from Table 1 that $-\Delta F$ is 600—800 cal/mol per CH_2 residue of the detergent. The value agrees with 700 cal/mol per CH_2 residue, which is obtained when CH_2 is transferred from the aqueous phase to the organic solvent.[11]

Literature survey[10,12-14] indicates that the free energy change $-\Delta F$ is larger when the anionic detergent (with sulfate or sulfonate) or fatty acid is bound to BPA than when the cationic detergent is bound, if the length of the hydrocarbon chain is the same in the range C_{10}—C_{16}. Tanford[15] has pointed out that both the ionic and hydrophobic interactions take part in the anionic detergent binding at pH 6.8, and attributed half of $-\Delta F$ to the ionic interaction. If $-\Delta F$ for the anionic detergent with C_{12} is compared with that for the cationic detergent with C_{12}, there is a difference by 3—4 kcal, which is almost the same as $-\Delta F$ estimated for ionic interaction.[15] Thus, it is concluded that the contribution of the ionic interaction is smaller in the cationic detergent binding than in the anionic detergent binding. In other words, the hydrophobic bonding is the main force in the cationic detergent binding.[16]

d) Cooperative Binding: It is clear in Fig. 1 that the cooperative binding also depends on the length of the hydrocarbon chain of the detergent. The change in the standard free energy accompanying the cooperative binding is given by the equation $\Delta F=RT \ln X$, where X is the mid-point of the two extreme equilibrium concentrations for the region in which the cooperative binding occurs.[15] The value of $-\Delta F$ was calculated for TTAB and DTAB at 25°C using the equilibrium concentration at which 85 detergent ions were bound.[17] The highest number of detergent ions bound was 170 for these two detergents (Fig. 1). It was found that $-\Delta F$ was 2.9 kcal/mol for DTAB binding and that it was 4.2 kcal/mol for TTAB binding. These figures lead to the conclusion that the free energy change was 650 cal/mol per CH_2 residue; the value is almost the same as that for the statistical binding. Thus, the hydrophobic bonding is also the main force in the cooperative binding.

e) Binding Isotherm at the Isoionic Point: The binding isotherm of the system BPA–DTAB at 25° C and pH 5.2 (in 0.1M KCl) was the same as that at pH 6.9; $r_{s,max}$ was 5 and k_0 was also the same within the experimental error. When r was less than 70, the isotherm at pH 5.2 lay on that at pH 6.9. When it

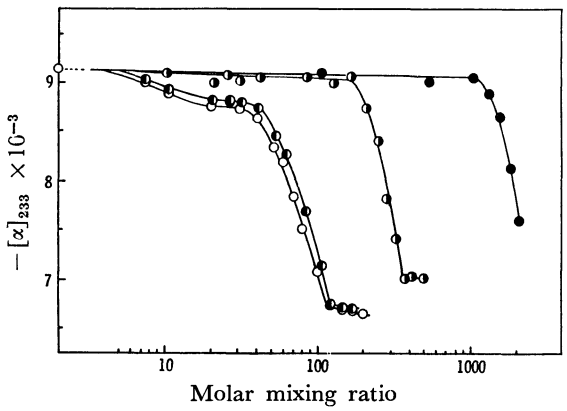

Fig. 3. $-[\alpha]_{233}$ *vs.* molar mixing ratio (detergent/BPA). pH 5.2 in 0.1 M KCl and 25 °C. ○: HTAB-BPA, ◐: TTAB-BPA, ◑: DTAB-BPA, ●: DeTAB-BPA.

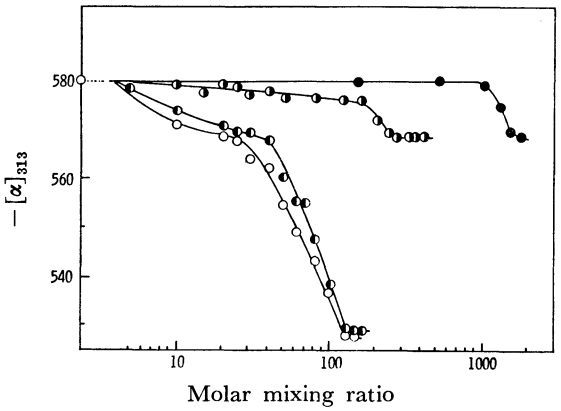

Fig. 4. $-[\alpha]_{313}$ *vs.* molar mixing ratio (detergent/BPA). pH 5.2 in 0.1 M KCl and 25 °C. ○: HTAB-BPA, ◐: TTAB-BPA, ◑: DTAB-BPA, ●: DeTAB-BPA.

was more than 70, the isotherm at pH 5.2 located upper than that at pH 6.9. The highest number of the detergent ions bound was slightly less, *i. e.*, 140.

Optical Rotation. a) Unfolding of BPA by Cationic Detergent at pH 5.2: Optical rotations were measured at the isoionic point of BPA in 0.1 M KCl (pH 5.2). Values of $-[\alpha]_{233}$ and $-[\alpha]_{313}$ are given as a function of the molar mixing ratio (detergent/BPA) in Figs. 3 and 4, respectively. It is clear in these figures that the BPA is disorganized by the binding of particular numbers of the detergent. Figure 3 shows how the helix content[18] in BPA decreases by the binding of the detergent. Detergent with higher carbon number is more effective in the range C_{10}—C_{14}; TTAB and

HTAB seem to have almost the same ability to unfold the BPA. Figure 4 shows how the tertiary structure[19] of BPA is disorganized by the cationic detergent.

In Figs. 3 and 4, it is seen that $-[\alpha]_{233}$ and $-[\alpha]_{313}$ are constant in the region of TTAB/BPA=20—40. If the data of equilibrium dialysis are combined, the complexes formed in this region are found to be AD_5—AD_{15} (A: BPA and D: cationic detergent). When the molar mixing ratio exceeds 40, both of the rotations decrease remarkably and continuously, attaining to a constant. Since $-[\alpha]_{233}$ is 6700 at the molar mixing ratio 120, *i.e.*, for AD_{60}, it is found that the helix of BPA was destructed by 40% by binding of 60 cationic detergents.[20] In the region of HTAB/BPA=10—25, $-[\alpha]_{233}$ and $-[\alpha]_{313}$ are constant. The compositions of the complexes are AD_8—AD_{20}. When the mixing ratio exceeds 25, values begin to decrease.

For DTAB, $-[\alpha]_{233}$ begins to decrease at AD_7 and attains constant at AD_{40}: $-[\alpha]_{313}$ drops at AD_7 and attains constant at AD_{20}. This means that the helix content of BPA decreases, keeping the tertiary structure constant, when r is more than 20. Almost the same is true for DeTAB. When 7 DeTAB are bound to BPA, both the helical content and the tertiary structure begin to decrease.

In conclusion, the ability for the cationic detergent to destruct BPA is in the order: HTAB≧TTAB>DTAB>DeTAB.[21]

b) Unfolding of BPA at pH 6.9 and 9.0: Changes in $-[\alpha]_{233}$ and $-[\alpha]_{313}$ with the molar mixing ratio TTAB/BPA were measured for SH blocked BPA at pH 6.9 and 9.0. Results are given in Fig. 5 by the expression $-[\alpha]_{233}$ *vs.* $-[\alpha]_{313}$. Measurements were made using SH blocked BPA at these pH's to prevent the SH–S-S exchange reaction.

Difference Spectra. In Fig. 6 are shown difference spectra of SH blocked BPA in presence of TTAB at pH 6.9. When the concentration of TTAB is low, peaks are observed at 275, 283, 292, and 298 nm, and troughs at 277, 287, 295, and 305 nm, *i.e.*, the red shift occurs. Especially, peak at 292 nm is large. Peaks increase their magnitudes with the increase in the amount of TTAB. Peak at 292 nm is caused by Try, those at 275 and 283 nm are caused by Try and Tyr, and peak at 298 nm would be caused by Try. The environment of Try residue, and probably that of Tyr residue also, seem to change when some detergents are bound to BPA.

Generally, the blue shift occurs when the protein is denatured, or when the buried chromophore in the protein is exposed to the aqueous solvent. The binding of alkyl sulfate, whose carbon number is more than 12, to BPA causes conformational change in BPA. The blue shift of peak at 292 nm (Try residue) occurs when smaller number of detergents are bound, and the blue shift of peak at 288 nm (Tyr residue) occurs when larger number of detergents are bound.[22,23] In the present study, however, the red shift was observed in a wider range of molar mixing ratio. This suggests that the cationic detergent unfolds BPA in such a way as to prevent the Try residue from exposing to the aqueous phase.

The same red shift was observed also at the isoionic point.

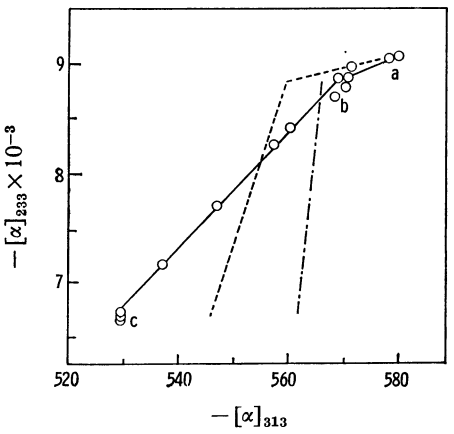

Fig. 5. Relations between $-[\alpha]_{233}$ and $-[\alpha]_{313}$. ○: BPA-TTAB complexes at pH 5.2 in 0.1 M KCl. a: AD_0, b: AD_{15}, and c: AD_{60}. (AD_x means the composition of the complex.) ----: SH blocked BPA-TTAB complexes at pH 6.9. —·—: SH blocked BPA-TTAB complexes at pH 9.

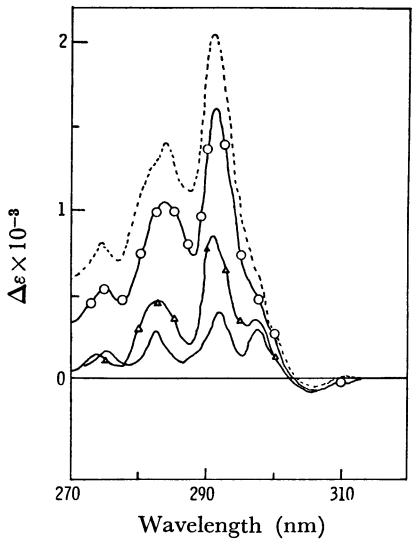

Fig. 6. UV difference spectrum of BPA-TTAB complexes. pH 6.9, ionic strength 0.1 and 25 °C. Sample: SH blocked BPA plus TTAB. Reference: SH blocked BPA. Molar mixing ratio are 20 (——), 30 (-△-), 50 (-○-) and 60 (·····).

General Discussion. As was described above, TTAB and HTAB were bound to BPA statistically up to $r=5$ at pH 6.9 and 5.2. When r was more than 5, the cooperative binding occurred accompanying a conformational change in BPA. Measurements of optical rotation revealed that the second unfolding begins to occur at $r=20$.

Studying the BPA solution by the small angle X-ray scattering, Luzzati *et al.*[24] suggested that the BPA is composed of the NH_2 terminal half which is compact and the COOH terminal half which is rather loose. Recently, Hilak *et al.*[25] supported this model by studying the fragments of BPA produced by the pepsin digestion and by measuring the optical rotation of each fragment in the N–F transition region. It is known that two Try residues are involved in the com-

pact NH_2 terminal half.[26] This time it has been found that the cationic detergent binding changes the environment of Try residue. This suggests that the compact NH_2 terminal half, at least, is unfolded by binding with the cationic detergent.

In the previous paper.[1] it was reported that the SH–S-S exchange reaction begins to occur between BPA molecules when 5 mol of TTAB are added to 1 mol of BPA at pH 9. In the present study, it has been found at pH 6.9 that the BPA is unfolded when r is more than 5. The BPA at pH 9 is unfolded by the N–B transition and by the binding of cationic detergent.[27]

The SH group is also in the compact NH_2 terminal half,[25] and locates in the crevice, its depth being at least 9.5 A.[29] If the BPA is unfolded, some of the S–S bond will be exposed,[30] and the crevice will be broken to expose the SH group. Thus the SH–S-S exchange reaction will take place easier at the alkaline pH in presence of the cationic detergent. In a recent study in our laboratory, it was deduced that the S–S bond is more reactive when it is surrounded by the cationic detergent.[32]

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16) Tanford[15] insisted that the free energy change for the interaction between BPA and the anionic detergent is constant when the carbon number of the detergent is more than 10. His explanation for the interaction is that the hydrophobic part of the detergent binds to a hydrophobic surface patch or patches of BPA. In contrast, the free energy change for the present interaction increased with the increase in the carbon number of cationic detergent up to the carbon number 16. Thus the above explanation by Tanford can not be applied. Our explanation is that the hydrocarbon part of the cationic detergent is buried into the hydrophobic crevice of BPA.
17) The equilibrium concentration of the detergent at the highest number of binding is equal to its critical micelle concentration (CMC) in phosphate buffer of pH 6.9, ionic strength 0.1 and 25 °C. The CMC is 1.6×10^{-3} mol/l for TTAB and 1.2×10^{-2} mol/l for DTAB. When the equilibrium concentration of the detergent is higher than the CMC, the micelle is formed in the solution.
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20) In Fig. 3, it is seen that the conformational change of BPA by TTAB finishes almost completely at AD_{60}. However, the complex AD_{60} is formed around the mid-point of the binding isotherm in the region of cooperative binding in Fig. 1. This means that binding of more than 60 TTAB does not induce additional unfolding in BPA. The same is true for other detergents. A similar result to this was obtained by Nozaki *et al.*[4]
21) Raynolds *et al.*[10] studied the conformational change of BPA caused by a series of anionic detergents whose carbon numbers are different. They stated that the detergent whose carbon number is less than 10 binds to BPA cooperatively without the conformational change in BPA, and that the detergent whose carbon number is more than 12 binds to BPA cooperatively inducing the conformational change in BPA. In the present study, cationic detergents induced the conformational change, when the carbon number was more than 10.
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27) When TTAB is added to BPA at pH 9.0, $-[\alpha]_{233}$ changes remarkably and $-[\alpha]_{313}$ changes slightly, giving almost perpendicular straight line without a break in Fig. 6. This is because the BPA in the B form unfolds.[28]
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