Biopolymer–Surfactant Interaction 2: Kinetics of Binding of Cetyltrimethylammonium Bromide with Bovine Serum Albumin

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The results of the kinetics of binding of the surfactant cetyltrimethylammonium bromide (CTAB) with bovine serum albumin (BSA) under varied conditions of CTAB concentration, pH, ionic strength and temperature are presented. The kinetic process has been found to be comprised of either two or three stages; the rate constants of the first, second, and third stages follow the general order of $k_1 \gg k_2 > k_3$. The three-stage kinetic process has been observed at higher CTAB concentrations, higher temperature and in presence of the protein denaturant urea. The activation parameters for this three-stage kinetic process have been determined. Most entropies of activation have been found to be negative (a very few are positive) whereas the activation enthalpies and energies are both positive and negative. The enthalpy and entropy for all the kinetic stages in different environmental conditions have been found to compensate each other, whereas the free energy of activation has remained unaffected. A model for the three-stage kinetic process has been proposed.

Surfactant–protein interaction can represent a good model for lipid-protein interaction existing in cell membrane.[1—3] Besides, the use of surfactants in biological, biochemical, and pharmaceutical works very often encounter interactions with biopolymers viz., proteins, enzymes or DNA.[3—15] The physicochemical investigations of surfactant–biopolymer interactions are, therefore, of fundamental and applied importance.

In recent years[9,10,16—19] equilibrium studies of such interaction have been stressed and a physicochemical basis for them has been proposed. For an understanding of the mechanism of such interactions leading to the equilibrium situation, exploration of kinetics of such processes is essential. Such studies have been very rare for want of a suitable physical method of probing. In a previous article,[20] this difficulty has been greatly overcome by the employment of a surfactant ion-selective membrane. The cetyltrimethylammonium ion-selective membrane electrode has been found to be an excellent device for continuously monitoring the decrease in the surfactant concentration due to its binding with the Na-salt of the biopolymer carboxymethyl cellulose (CMC). The method has been established to be simple, efficient and reproducible; calibration of the membrane electrode against known concentrations of CTAB helps to accurately determine the unknown concentration of the surfactant. According to our literature survey, the work done is the first of its kind and therefore, original and unique.

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This paper presents a detailed study of the kinetics of the interaction of cetyltrimethylammonium bromide (CTAB) with bovine serum albumin (BSA) under various conditions of surfactant concentration, pH, ionic strength and temperature. The activation parameters for the kinetic process of CTAB–BSA interaction have been evaluated and analysed. The effect of urea (the protein denaturant) medium on the kinetic process has also been investigated for the understanding of the binding kinetics which has been found to be complex.

Experimental

Materials. Pure crystalline bovine serum albumin (BSA) used for this kinetic study was obtained from Sigma Chemical Co., USA. CTAB used was also a Sigma product used earlier.[20] All inorganic salts used were of analytical grade. Urea used was obtained from British Drug House, UK, and recrystallised from alcohol. Tetrahydrofuran used for membrane preparation was purchased from S.D. Fine Chemicals, India. Doubly distilled conductivity water was used throughout the experimental work.

The membrane was prepared by dissolving poly(vinyl chloride) (PVC) and the carrier complex CTA–DC (cetyltrimethyl ammonium bromide complexed with sodium deoxycholate) in tetrahydrofuran using dioctyl phthalate as plasticizer. The mixture was spread on mercury surface to form a thin film after evaporation of the solvent. Further details about the preparation are available in our earlier publication.[20]

Methods. The surfactant concentrations at different time intervals were measured setting a CTAB ion-selective membrane electrode similar to that described by Hayakawa and Kwak,[21] and adapted in our conditions. The electrode assembly was as follows:

Calomel electrode/Test solution/Membrane/Ref. solution//Calomel electrode.

The reference solution containing 1 mmol dm^{-3} CTAB in 10 mmol dm^{-3} NaCl was taken inside a PVC tube, to one end of which the membrane was attached. The test solution outside the tube contained CTAB of the order 10 μmol dm^{-3} and BSA of the order of 3 μmol dm^{-3}.

The membrane potential arising from the difference in concentrations of the two solutions was measured with a digital multimeter of Hindustan Instruments Limited, Hyderabad, India with an accuracy of ±0.1 mV. Membranes showing quick Nernstian response and stability were employed in the kinetic experiments. The test solution was constantly stirred with a magnetic stirrer during the kinetic study. After the protein solution was added to the test solution with the help of a Hamilton microsyringe, the surfactant concentration in solution decreased due to the binding of CTAB with BSA and hence the emf values started changing with time.

An emf–concentration calibration curve was initially constructed with known [CTAB] values, from which the surfactant concentrations at different time intervals were calculated. The emf reaches a stable value a short interval of time after the addition of the surfactant in the test solution (Fig. 1). The diagramatic representation of the experimental assembly is depicted in Fig. 2. The experimental set-up was thermostated in a water-bath of ±0.02 °C uncertainty.

The kinetic study of the surfactant binding was carried out at different pH values, ionic strengths, surfactant concentrations and temperatures. The experiment was also performed in the presence of 4 mol dm^{-3} urea.

A constant concentration of BSA (3 mol dm^{-3}) was used throughout the experiment. Increased concentration (tenfold) of BSA was found not to affect the kinetic results and membrane behavior.

A perceptible change was observed in presence of [NaCl]$\geqslant$0.5 mol dm^{-3}. The thermodynamic property of the membrane electrode was, therefore, considered to be practically independent of [BSA] and [NaCl].

Results and Discussion

Kinetic Course and Reaction Order. Bovine Albumin dissolved in aqueous solution behaves as rigid globular macromolecules whose boundary region is extensively hydrated both in the presence and absence of neutral salts in the medium. The study of equilibrium dialysis shows[18,22–24] that the protein binds with the long chain cationic surfactant CTAB under different conditions of pH, ionic strength, and temperature.

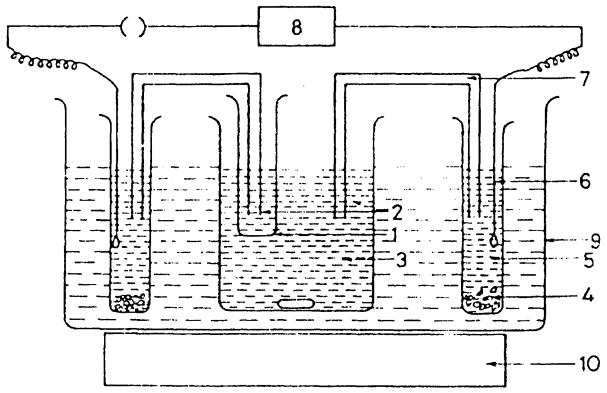

Fig. 2. A schematic representation of membrane potential measurements. 1—PVC membrane electrode; 2—reference solution,; 3—test solution; 4—solid KCl; 5—saturated KCl solution; 6—Calomel electrode; 7—salt bridge; 8—multimeter; 9—temperature bath; 10—magnetic stirrer.

In the present study, binding ratios of CTAB to BSA at a given total surfactant concentration C_s have been determined as a function of the elapsed time, t, keeping pH, ionic strength, and temperature constant. The variation of the amount of surfactant $(C_0 - C_t)$ (where C_0 and C_t are surfactant concentrations present initially and at time t, respectively) bound to BSA with time at different surfactant concentrations is shown in Fig. 3. In each case, $(C_0 - C_t)$ is observed to increase sharply with increase of t from about five seconds to higher values, until the equilibrium value of the binding is attained. One also finds that the initial slopes of the $(C_0 - C_t)$ vs. t plot increases with increase of C_s. If it is assumed that the biocolloid particles suspended in the aqueous medium form a two-phase system, then the initial kinetic process occurring in the extremely low range of elapsed time is diffusion-controlled. The adsorption of surfactants at the solid–liquid interfaces has been amply demonstrated. If we assume that interaction of CTAB with proteins occurs in a single phase, then following the law of mass action, it can

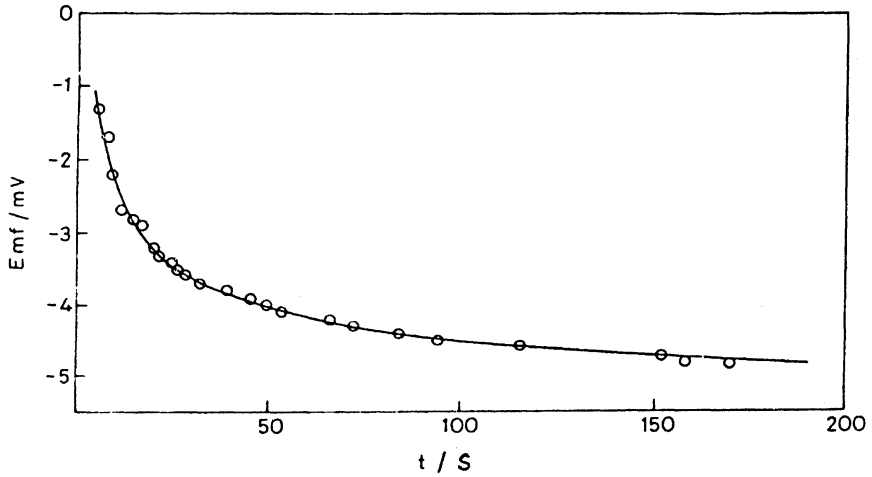

Fig. 1. Emf. vs. time plot at pH = 3.8; μ = 0.1, and [CTAB] = 30 μmol dm^{-3} and T = 301 K.

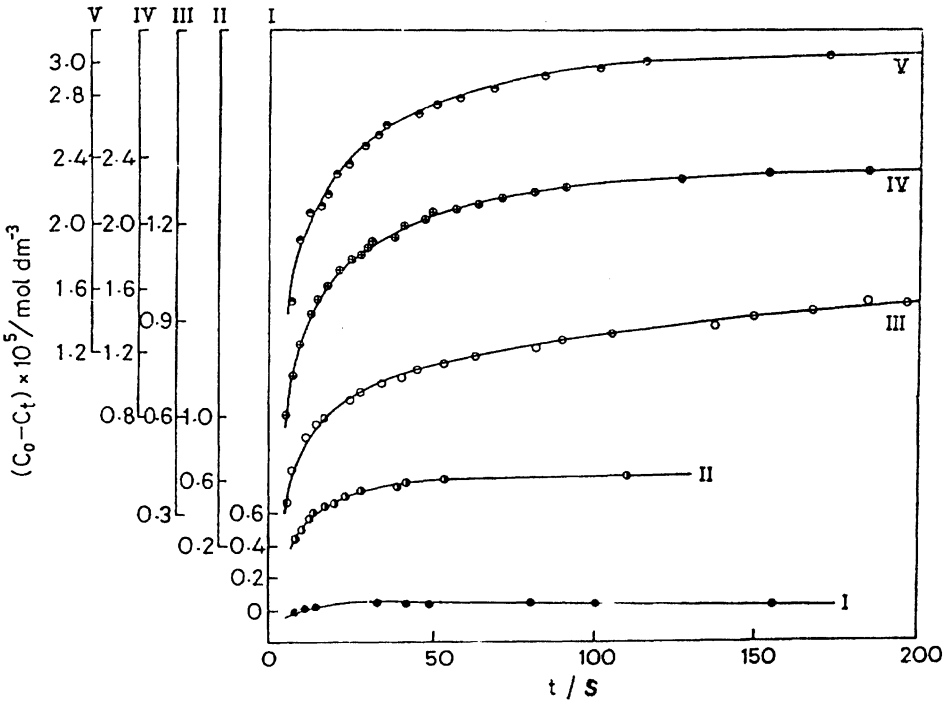

Fig. 3. The extent of CTAB bound with BSA vs. time t profiles at $T=310$ K; $\mu=0.05$; pH = 5.0. The ordinates scale is indicated in the plot. ●, ◑, ○, ⊗, ◓ represent [CTAB] 10, 30, 50, 80, and 100 μmol dm^{-3}, respectively.

be expected that the initial slope should also increase with increase of C_s in the medium. The kinetic courses at 0.2% (w/v) BSA at [CTAB] = 10, 30, 50, and 100 μmol dm^{-3} and (ionic strength) = 0.05 at 301 K are presented in Fig. 3, the ordinate representing the amount of bound CTA^+ ions. They resemble CTAB–CMC (carboxymethylcellulose) interaction and protein denaturation patterns.[20,25,26] An initial rapid binding kinetics lengthened and sharpened by increased [CTAB] was observed. The substrates are under the constant binding influence of the site.

To process the kinetic data the number of moles of CTA^+ ions bound per mole of protein (Γ_s) has been evaluated from the relation

$$\Gamma_s = \frac{(C_0 - C_t)vM}{1000w}, \qquad (1)$$

where v is the volume of the solution, w is the mass of BSA, and M is its molar mass.

The C_t values obtained from the emf vs. log [CTAB] calibration plots (not shown) were used in Eq. 1 to estimate Γ_s. The equilibrium (maximum) binding of CTA^+ to BSA i.e. Γ_s^e was estimated from the isotherms whose typical representations are shown in Fig. 3. Like the CTA^+–CMC binding kinetics, the CTA^+–BSA interaction process was considered to follow first-order kinetics: More than one pseudo-first-order rate constant (two or three) were also anticipated. The results were analyzed following the suggestion of Davis.[27] An analytical relation of the following type was used:

$$\Gamma_s = \Gamma_s^e - \Gamma_s^1 e^{-k_1 t} - \Gamma_s^2 e^{-k_2 t} - \Gamma_s^3 e^{-k_3 t}, \qquad (2)$$

where the new terms, k_1, k_2, and k_3 are the pseudo-first-order rate constants for a three-stage kinetic process with Γ_s^1, Γ_s^3 as the respective concentrations of maximum CTA^+ that may undergo interaction with BSA at appropriate stages of the process such that

$$\Gamma_s^e = \Gamma_s^1 + \Gamma_s^2 + \Gamma_s^3.$$

The fourth term in Eq. 2 does not contribute, if the kinetics is a two-stage process. A least-square curve-fitting procedure was used to calculate the rate constants in a computer. The fitting patterns of three experimental runs under varied conditions are presented in Fig. 4.

The results obtained at different [CTAB] at pH=5, μ=0.05, and at different temperatures are presented in Table 1. Those at constant [CTAB] of 30 mol dm^{-3}, but at different pH and temperature, are given in Table 2. The membrane potential at the state of binding equilibrium has a maximum uncertainty of ±5%. This imparts maximum uncertainties in k_1, k_2, and k_3 to the extents of ±2%, ±4% and ±6% respectively.

Table 1 reveals that $k_1 \gg k_2 > k_3$ at all temperatures and that their dependence on [CTAB] at the studied temperatures is not systematic. At higher temperature, the three categories of k are virtually independent of [CTAB]. While only two types of rate constants, k_1 and k_2, are obtained at lower [CTAB] and lower temperature, three rate constants were realized at higher temperature. At constant [CTAB], no general trends in the rate constant with μ and pH were observed (Table 2). The kinetic process, comprised only two stages at all μ and pH values for 292.9 and 301 K. The occurrence of three stages took place at the highest studied temperature (318 K) except at the highest pH=6.2. The results presented in Tables 1 and 2 always maintain the trend $k_1 \gg k_2 > k_3$, where k_1 is on the average 6—10 times higher than k_2. The first stage is distinctly different from the subsequent stages.

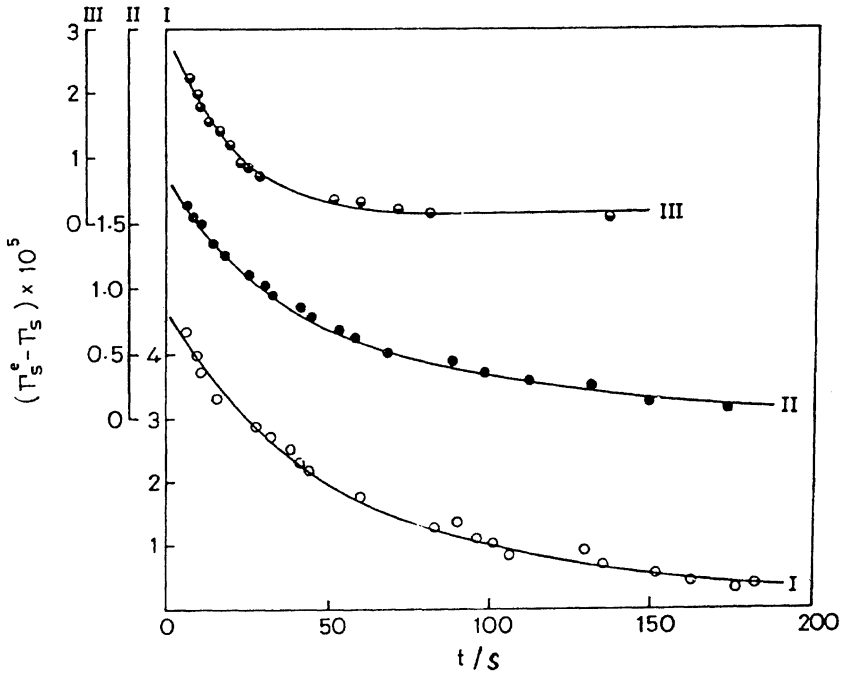

Fig. 4. $(\Gamma_s^e - \Gamma_s) \times 10^5$ (mol CTA^+)/(mol BSA) vs. time t plot for CTA^+ binding with BSA, ○, ●, ◒ represent experimental points, curves represent theoretical fit. Curve I represent binding in presence of 4 (M) urea at T=301 K, μ=0.05, pH=5.0, and [CTAB]=100 μmol dm^{-3}. Curve II represents binding at T=310 K, μ=0.1, pH=6.2, and [CTAB]=30 μmol dm^{-3}. Curve III represents binding at T=318 K, μ=0.05, pH=5.0, and [CTAB]=50 μmol dm^{-3}.

Table 1. Rate Constants of BSA–CTAB Interaction at Different [CTAB] and Temperature at pH=5 and μ=0.05

Temp	[CTAB]	k_1	k_2	k_3	±STD
K	mol dm^{-3}	h^{-1}	h^{-1}	h^{-1}	
	10	238	28.8		0.271
292.4	30	414	32.4		0.338
	50	360	36.0		0.180
	80	335	72.0	3.6	0.275
	100	396	63.0	7.2	0.366
	10	358	54.0		0.035
301	30	364	21.6		0.247
	50	407	36.0		0.277
	80	342	32.9	10.8	0.484
	100	454	36.0	9.0	0.376
	10	299	43.2		0.485
	30	317	50.4		0.361
310	50	367	47.9	12.6	0.248
	80	363	48.6	18.0	0.478
	100	407	43.9	10.1	0.444
	10	353	37.2	25.2	0.379
	30	338	40.7	28.8	0.258
318	50	331	57.6	32.4	0.367
	80	385	39.6	23.4	0.478
	100	353	46.8	29.2	0.339

Energetics of the Activation Process. The activated complex between CTA^+ and BSA leading to a stable product is according to Eyring's postulate:

$$\underset{}{\text{BSA} + \text{CTA}^+ \rightleftarrows} \underset{\text{Activated Complex}}{(\text{BSA–CTA}^+)} \rightarrow \underset{\text{Product}}{\text{BSA–CTA}^+}. \quad (3)$$

In the linear form, the rate equation reads:

$$\ln \frac{k}{T} = \left(\ln \frac{k}{h} + \frac{\Delta S^*}{R}\right) - \frac{\Delta H^*}{RT}, \quad (4)$$

where, ΔH^* and ΔS^* are the enthalpy and entropy of activation, respectively, and the other terms have their usual significance. The linear plot between $\ln k/T$ and T^{-1} should yield ΔH^* and ΔS^* from the slope and intercept, respectively. For such linear behavior, however, it is a primary requirement that k should either increase or decrease with increasing temperature. As already stated, no such regular behaviour has been observed for any set of experiments. The Eyring equation at two temperatures, T_1 and T_2, was thus combined in the following form:

$$\ln\left(\frac{k_1}{T_1} / \frac{k_2}{T_2}\right) = \frac{\Delta H^*}{R}\left(\frac{1}{T_2}\right) - \frac{1}{T_1}, \quad (5)$$

with the assumption that ΔH^* and ΔS^* remain constant when T_1 is close to T_2, with k_1 and k_2 being the respective rate constants. In fact, these parameters refer to the average temperature T_{av} equal to $\frac{1}{2}(T_1+T_2)$. The values of ΔH^* and ΔS^* estimated in this manner in terms of Eq. 5 for different systems are presented in Tables 3 and 4. The values of the free energies of activation (ΔG^*) have been also evaluated from the relation: $\Delta G^* = \Delta H^* - T\Delta S^*$, and are included in the tables.

The data analysis herein followed to quantify the energetics of the kinetic process is based on the validity of Eqs. 4 and

Table 2. Rate Constants for BSA–CTAB Interaction at [CTAB]=30 μmol dm^{-3} at Different pH, μ, and Temperature

Temp / K	pH	μ	k_1 / h^{-1}	k_2 / h^{-1}	k_3 / h^{-1}	±STD
	3.8	0.01	360	61.4	—	0.273
		0.05	357	54.0	—	0.247
		0.1	353	68.4	—	0.478
292.4	5.0	0.01	346	72.0	—	0.165
		0.05	414	32.4	—	0.180
		0.1	407	58.3	—	0.433
	6.2	0.01	414	59.4	—	0.474
		0.05	400	57.6	—	0.333
		0.1	324	36.0	—	0.303
	3.8	0.01	367	36.3	—	0.276
		0.05	310	39.6	—	0.315
		0.1	259	42.5	—	0.295
301	5.0	0.01	245	50.4	—	0.328
		0.05	364	21.6	—	0.280
		0.1	263	43.2	—	0.328
	6.2	0.01	436	57.6	—	0.007
		0.05	428	59.0	—	0.010
		0.1	410	55.8	—	0.015
	3.8	0.01	392	48.6	—	0.010
		0.05	428	41.4	14.4	0.017
310		0.1	410	46.1	18.0	0.014
	5.0	0.01	421	43.9	—	0.008
		0.05	317	50.4	—	0.248
		0.1	540	54.0	—	0.022
	6.2	0.01	468	55.8	—	0.013
		0.05	540	57.6	—	0.330
		0.1	482	68.4	24.5	0.030
	3.8	0.01	320	28.8	10.8	0.343
		0.05	346	30.6	15.1	0.157
		0.1	396	29.9	18.4	0.026
318	5.0	0.01	407	28.8	16.2	0.265
		0.05	338	40.7	28.8	0.367
		0.1	400	39.6	20.2	0.021
	6.2	0.01	374	54.0	—	0.284
		0.05	382	52.0	—	0.476
		0.1	353	41.1	—	0.347

5. The reported k values have a nonsystematic dependence on temperature as well as on [CTAB] and μ. This we attribute to the complex nature of the binding kinetics of CTAB with BSA and not to the uncertainties (errors) of measurements. The orderly behavior of the kinetic process with respect to temperature in 4 mol dm^{-3} urea medium (making Eq. 4 valid for data analysis) coroborates this view. Our previous results on CTAB–CMC binding kinetics realized under similar conditions of measurement have shown systematic variations of k with temperature, [CTAB], etc. giving further support to the above view. We add that temperature dependence of k takes care of the strength of interaction guided by site modification, structural change, etc. affecting the activation complex; the energetic parameters include the contributions from all these effects. This is inevitable for substrate binding process to complex macromolecules viz., proteins, enzymes, etc. Between two close temperatures, Eq. 5 has been considered valid; the nonsystematic activation parameters suggest that the formation and stabilisation of the activated complex are variable functions of temperature.

Examination of the thermodynamic parameters show that ΔG_1^*, ΔG_2^*, and ΔG_3^*, representing free energy changes for the activated complex formation, are all positive and are not significantly different from one another. The corresponding entropies of activation (ΔS_1^*, ΔS_2^*, and ΔS_3^*) in most cased (except a few) are negative; the formed activation complexes are therefore organized. Further, ΔS^* values in a number of cases are positive since $\Delta G^* > T_{av}\Delta S^*$, in other cases $\Delta G^* < T_{av}\Delta S^*$, so that ΔH^* values become negative. The latter is caused by the high magnitudes of ΔS^*. Since the ΔG^* values are found to be close in all cases, ΔH and $T_{av}\Delta S^*$ compensate each other under the studied physico-chemical conditions; the system has therefore shown an isokinetic effect.[28]

Kinetics in Aqueous Urea Medium. The kinetics of CTAB interaction with BSA and its activation parameters have also been studied in the presence of 4 mol dm^{-3} of urea, an established protein denaturant.[29] The results are presented in Tables 5 and 6. Fitting of experimental results at μ=0.05, pH=5, and temperature 301 K at [CTAB]=100 μmol dm^{-3} is presented in Fig. 4 (Curve 1). Three-stage binding kinetics has been observed in the urea medium. The normal trend of $k_1 \gg k_2 > k_3$ is observed and all the constants have increased with temperature. The rate constants are lower than those without urea. The energetic parameters obtained from Eyring plot (Fig. 5) in 4 mol dm^{-3} urea also have shown comparable behaviors with those without it. All the ΔH^* values are considerably lower than aqueous medium; the entropy values are equally negative. The ΔG_1^* and ΔG_2^* are close but the ΔG_3^* value is appreciably higher. It is thus observed that the denatured BSA has brought the kinetic events more in order. The results presented show that the three-stage kinetic process occurs at higher surfactant concentration, at higher temperature and in urea medium. This suggests initiation of partial denaturation of BSA by the surfactant CTAB at higher concentration. Auxillary measurements are necessary to support this. We will include them as a part of our future work plan. It may be added that intrinsic viscosity measurements of BSA in presence of CTAB have evidenced an increase of [η] from 3.8 to 8.2 ml g m^{-1} at 303 K; enlargement of the globular form of BSA is supported.

Probable Model for the Three-Stage Kinetics. Molecules of BSA are known to be ellipsoid (prolate) in shape, with an axial ratio close to 3.5. The isopiestic experiments have shown that there exists one or two layers of water bound with the protein in the aqueous medium with varied degrees of the energy of hydration.[30] It is considered that the hydrophobic, ionic and nonionic (hydrophilic) groups associated with the rigid molecule in the boundary as well as in

Table 3. Activation Parameters for BSA–CTAB Interaction at Different [CTAB] and Temperature at pH=5 and μ=0.05

Average temp/K	[CTAB] μmol dm^{-3}	ΔH^*/kJ mol^{-1}			ΔS/J K^{-1} mol^{-1}			ΔG/kJ mol^{-1}		
		ΔH_1^*	ΔH_2^*	ΔH_3^*	ΔS_1^*	ΔS_2^*	ΔS_3^*	ΔG_1^*	ΔG_2^*	ΔG_3^*
298.7	10	32.1±2	50.8±4	—	89±7	43±3	—	58.6±4	63.5±5	—
	30	−13.4±1	−36.8±2.5	—	−241±15	−342±22	—	57.8±4	64.5±4.5	—
	50	7.9±0.5	66.2±4.5	—	−169±12	4.6±1.5	—	57.9±5	64.8±4.5	—
	80	−0.6±0.1	−68.9±5	90.8±6	−198±14	−445±32	76±7	58.3±5	63.1±4.5	68.1±6
	100	9.1±0.8	−49.9±4	16.1±1	−164±13	−381±26	−173±10	57.7±3	63.1±5	67.5±6
305.5	10	−18.1±1	−21.8±1.5	—	−256±18	−284±20	—	60.2±5	64.9±5	—
	30	−14.5±1	70.8±5	—	−244±16	15.8±2	—	60.1±5	66.0±5.5	—
	50	−11.4±1	21.8±1.5	—	−237±15	−143±12	—	60.9±4	65.4±5.5	—
	80	2.6±0.2	31.2±2	41.6±4	−188±14	−112±10	−87±7	60.0±5	65.5±5	68.2±6
	100	−11.9±0.9	14.9±1	7.8±1	−234±15	−165±13	−200±16	59.5±5	65.5±5	69.1±5.5
314	10	14.3±0.9	−17.8±1	—	−152±13	−271±20	—	61.9±4	67.4±6	—
	30	4.1±0.2	−23.7±1.5	—	−184±14	−289±23	—	61.9±5	67.1±5.5	—
	50	−12.9±1	−42.2±3	93.3±6.5	−238±15	−349±26	77±7	61.7±4.5	67.4±6	69.2±5
	80	3.4±0.2	−23.3±2	−23.3±1.5	−185±15	−289±20	−146±11	61.5±5	67.4±5.5	69.1±5.5
	100	−17.0±1	3.4±0.2	106.2±8	−250±16	−203±16	113±9	61.5±5	67.1±5.5	70.8±5.5

Table 4. Activation Parameters for BSA–CTAB Interactions at Different pH and Temperature at [CTAB]=30 μmol dm^{-3}

Average temp/K	pH	μ	ΔH^*/kJ mol^{-1}			ΔS^*/J K^{-1} mol^{-1}			ΔG^*/kJ mol^{-1}		
			ΔH_1^*	ΔH_2^*	ΔH_3^*	ΔS_1^*	ΔS_2^*	ΔS_3^*	ΔG_1^*	ΔG_2^*	ΔG_3^*
296.7	3.8	0.01	−0.8±0.1	−46.9±4	—	−198±15	−371±22	—	58.1±5	63.2±5.5	—
		0.05	−14.4±1	−28.7±3	—	−245±18	−310±21	—	58.3±5	63.2±5.5	—
		0.1	−28.6±1.5	−42.8±3	—	−294±22	−356±22	—	58.5±5	62.8±5	—
	5.0	0.01	3.1±0.1	22.1±2	—	−185±17	−139±11	—	59.7±5	64.7±6	—
		0.05	25.0±3	1.3±0.2	—	−112±10	−210±16	—	59.1±4.5	65.4±6	—
		0.1	36.4±3.5	4.4±0.3	—	−74.3±7	−199±15	—	59.1±5	65.1±5.5	—
	6.2	0.01	−19.4±2	−46.6±4	—	−262±21	−231±18	—	62.8±55	69.0±6	—
		0.05	−20.4±2	−27.7±3	26.5±2	−265±22	−309±22	−134±11	62.7±5	69.3±6	68.5±5.5
		0.1	−5.2±0.2	−38.9±3.5	0.3±0.02	−213±20	−347±21	−222±17	61.7±5	69.9±6	69.4±6
305.5	3.8	0.01	−30.4±5	−31.5±3	—	−300±23	−317±23	—	58.7±5	62.5±5	—
		0.05	−12.2±1	−35.3±3	—	−237±18	−337±23	—	57.9±5	64.6±5.5	—
		0.1	−15.2±1	−26.6±2.5	—	−247±19	−302±20	—	58.0±5	63.0±5.5	—
	5.0	0.01	44.4±4	−14.5±1.5	—	−51.7±4	−26.0±2	—	60.2±5	65.1±5.5	—
		0.05	−15.5±1	−30.3±3	—	−244±20	−314±25	—	60.0±5	65.4±6	—
		0.1	36.3±3	16.8±2	—	−75.9±6	−158±13	—	59.5±5	65.0±6	—
	6.2	0.01	−6.1±0.4	−45.4±4	—	−215±17	−360±26	—	61.3±5	67.7±6	—
		0.05	4.0±0.2	−24.3±2	—	−184±19	−291±24	—	61.9±5	67.1±6	—
		0.1	−33.1±3.5	−34.1±2.5	17.2±1	−300±22	−332±25	166±13	61.0±5	67.0±6	69.5±5.5
314	3.8	0.01	1.8±0.1	−5.1±0.2	—	−189±15	−228±17	—	57.7±4	62.6±5.5	—
		0.05	3.5±0.15	−2.9±0.1	—	−183±14	−221±19	—	57.8±4.5	62.6±5.5	—
		0.1	17.5±1	34.8±3	—	−137±13	−96±9	—	58.1±5	63.2±5.5	—
	5.0	0.01	3.7±0.1	−5.3±0.2	—	−182±15	−229±21	—	59.3±5	64.6±6	—
		0.05	17.5±1	−4.7±0.2	—	−137±12	−227±22	—	59.2±5	64.5±6	—
		0.1	11.5±0.9	15.1±1	—	−157±12	−162±12	—	59.3±5	64.4±6	—
	6.2	0.01	−25.2±2	58.8±5	—	−276±22	−231±18	—	61.3±5	66.5±5.5	—
		0.05	−37.8±3	−13.0±1.5	—	−315±22	−253±17	—	61.0±5	66.6±6	—
		0.1	−34.3±3	−54.3±5	—	−304±21	−388±22	—	61.3±5	66.7±6	—

Table 5. Rate Constants of BSA–CTAB Interaction in 4 mol dm^{-3} Urea at Different Temperatures at μ=0.05, pH=5.0, [CTAB]=100 μmol dm^{-3}

Temp/K	k_1/h^{-1}	k_2/h^{-1}	k_3/h^{-1}	±STD
292	230	36.0	5.4	0.127
301	252	37.1	7.5	0.330
310	292	39.3	10.1	0.257
318	306	43.2	14.4	0.275

the interior regions are distributed heterogeneously. There exists three well-defined domains of the protein molecule on the basis of distribution of the hydrophobic and hydrophilic groups of BSA in forming the rigid structure. Even these domains can be separated using various types of enzymes.[30] One end of the domain of the ellipsoid molecule appears to be highly charged while the other end of the domain is found to be highly hydrophobic in nature; the middle region is moderately hydrophilic.

We propose that affinities of CTAB molecules for different

Table 6. Activation Parameters for BSA–CTAB Interaction in 4 mol dm^{-3} Urea at 305.5 K, μ=0.05, pH=5.0, [CTAB]=100 μmol dm^{-3}

ΔH^*/kJ mol^{-1}			ΔS^*/J K^{-1} mol^{-1}			ΔG^*/kJ mol^{-1}		
ΔH_1^*	ΔH_2^*	ΔH_3^*	ΔS_1^*	ΔS_2^*	ΔS_3^*	ΔG_1^*	ΔG_2^*	ΔG_3^*
6.2±0.3	3.0±0.2	5.0±0.3	−199±16	−215±17	−230±20	66.9±6	68.7±6	75.3±6.5

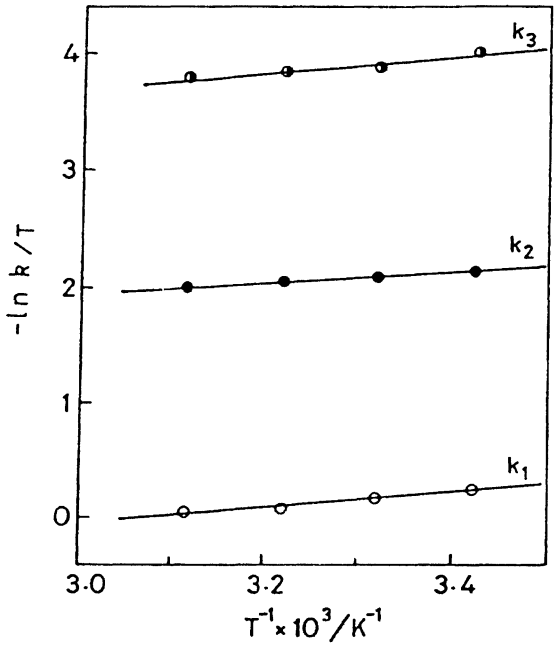

Fig. 5. $(-\ln k/T)$ vs. T^{-1} profile at [CTAB] = 100 μmol dm^{-3}, pH=5.0, μ=0.05, in presence of 4 (M) urea.

regions of the BSA molecules are different. The bound water molecules and highly charged (negative) domain of BSA in all probability are undergoing very fast interactions with CTAB due to high affinity and k_1 is associated with this process. This fast process becomes complete within a short span of time, following which the hydrophobic domain interacts with the hydrophobic part of CTAB; k_2 may be identified with the process. Finally, CTAB becomes involved with the central domain of BSA and the rate constant k_3 may be associated with this process, which is the slowest. For each step, both enthalpic and entropic contributions occur on a compensation basis during the formation of the activation complexes.

Conclusions

The results help to draw the following conclusions.

1. The interaction of CTAB with BSA follows first-order kinetics and takes place in two stages at lower temperature (290—310 K) and at low [CTAB]; it occurs in three stages at higher temperature and higher [CTAB]. In all conditions, the first-stage is much faster than the second.

2. Denatured BSA by urea is prone to show three-stage binding kinetics with CTAB.

3. Large negative entropy of activation in all conditions has supported the existence of a well-organized activated complex.

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