of *meso*-lanthionine was the sulfone (see Scheme III). Isolated *meso*-lanthionine sulfone was eluted in the amino acid chromatogram at about 35 min; thus it accounted for the peak that had been designated as unidentified (Lipton et al., 1977). This sulfone was previously reported by Zahn and Osterloh (1955).

Smith et al. (1974) identified *S*-methylcysteine sulfoxide as the toxic factor in kale and other brassica crops (Whittle et al., 1976) that causes hemolysis in ruminants. On the other hand, *S*-methylcysteine sulfoxide lowered the cholesterol in the plasma of rats (Itokawa et al., 1973). *S*-Methylcysteine sulfoxide was identified in cabbage (Synge and Wood, 1956). The high consumption of cruciferous crops in Japan (estimated human consumption of *S*-methylcysteine sulfoxide is 300 mg person^{-1} day^{-1}) has been suggested to protect against heart disease (see Whittle et al., 1976).

ACKNOWLEDGMENT

The author thanks Marguerite Kincius for help in the preparation of the manuscript and Albert H. Coleman, Jr. for advice and for review of the manuscript.

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Received for review April 6, 1978. Accepted July 10, 1978. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Effects of Ionic Dextran Derivatives on Heat Precipitation of Protein

Kunihiko Gekko* and Hajime Noguchi

The effects of concentration, ionic or hydrophobic group density, and molecular weight of ionic polysaccharides on the heat precipitation of bovine plasma albumin (BPA) were investigated. Carboxymethyldextran (Cm-dextran), carboxymethylbenzyldextran (Cm-Bzl-dextran), dextran sulfate, and chondroitin sulfate were used as ionic polysaccharides. The heat denaturation of BPA was promoted by all ionic polysaccharides added, but there existed a critical point in the dependence of concentration, charge density, and molecular weight of ionic dextran derivatives on the heat precipitation of BPA. The kinetic studies showed that the heat precipitation of BPA in the presence of Cm-dextran and Cm-Bzl-dextran is accelerated by activation enthalpy change rather than activation entropy change, but in the presence of dextran sulfate its acceleration is mainly caused by the large increase of activation entropy. These results were discussed from the viewpoint of the different interaction behaviors of these dextran derivatives with BPA molecule.

The interaction between proteins and polysaccharides has recently been shown to be an essential element in the study of food texture or processing (Morris, 1973). Such investigations can present much valuable information for utilization of polysaccharides in precipitation systems of proteins (Hildago and Hansen, 1969; Hill and Zadow, 1974, 1975). For example, the interaction between milk proteins and carboxymethylcellulose (Cm-cellulose) at pH 3–4 has been used as a means of precipitating whey proteins (Hansen et al., 1971), while at pH 4–5 the interaction has been used to prevent the precipitation of proteins in fruit-flavored milk drinks (Asano, 1966). The effect of polysaccharides on protein solubility may be analyzed as a sum of three contributions: (1) the excluded volume effect by polysaccharide itself, (2) the salting in or out effect by an electrostatic field of polysaccharide, and (3) the intermolecular interaction between protein and polysaccharide through the electrostatic force, hydrophobic bond, and hydrogen bond. When the solubility of protein decreases on thermal denaturation, though such a system is common in food processing, the effect of protein–polysaccharide interaction on protein stability may be important to its solubility. However, only a small amount of fundamental research (Elbein and Mitchell, 1975; Imeson et al., 1977) has been carried out in this field because the system is very complicated to analyze. A possible approach to analyze such complicated systems may be to estimate systematically each contribution as mentioned above. From these points of view, in the present paper, the effect of dextran derivatives on thermal precipitation of bovine plasma albumin is systematically investigated as a model system because the thermal denaturation of bovine plasma albumin (Levy and Warner, 1954; Warner and Levy, 1958; Štokrová and Šponar, 1963; Petersen and Foster, 1965a), the properties of dextran derivatives (Noguchi et al., 1973; Gekko and Noguchi, 1974, 1975), and the interaction between them (Gorter and Nanninga, 1953; Noguchi, 1956, 1960; Bettelheim et al.,

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0021-8561/78/1426-1409$01.00/0

Table I. Characteristic Properties of Ionic Polysaccharides

polysaccharide	sample no.	degree of substitution CH_2-COO^-	SO_3^-	CH_2-C_6H_5	mean degree of polymerization
dextran	dextran				240
carboxymethyldextran	CMD-1	1.62			7.7
	CMD-2	1.50			37
	CMD-3	1.49			68
	CMD-4	1.57			430
	CMD-5	1.63			1400
	CMD-6	0.35			240
	CMD-7	0.72			
	CMD-8	0.99			
	CMD-9	1.40			
	CMD-10	1.80			
	CMD-11	2.54			
carboxymethylbenzyldextran	CMBD-1	0.35		0.20	240
	CMBD-2	0.35		0.38	
	CMBD-3	0.35		0.50	
	CMBD-4	0.35		0.64	
dextran sulfate	DS-1		0.61		310
	DS-2		1.46		
chondroitin sulfate A	DS-3		2.54		
	CS-A	1.00[a]	1.00[a]		

[a] This value is calculated as the number of COO^- or SO_3^- group per repeating unit.

1966) have been well characterized.

EXPERIMENTAL SECTION

Materials. Crystallized bovine plasma albumin (BPA) was purchased from Nutritional Biochemicals Corporation (Cleveland, Ohio). Polyacrylamide disc electrophoresis of this material showed that there was one or two faint bands with slower mobilities in addition to the characteristic monomer and dimer bands. The BPA contained approximately 7% dimer or polymer as judged from the exclusion chromatography pattern.

Dextran sulfate containing various amounts of sulfate group and carboxymethyldextran (Cm-dextran) with different degrees of polymerization of approximately identical amounts of carboxyl group were kindly supplied by the Central Research Institute of Meito Sangyo Co., Ltd., Nagoya. Carboxymethylbenzyldextran (Cm-Bzl-dextran) and a part of Cm-dextran having various degrees of substitution were the same materials as used in the previous work (Gekko and Noguchi, 1975), which were prepared by etherification of purified dextran (Meito Sangyo Co., Ltd., mol wt 38000). Chondroitin sulfate A (prepared from whale cartilage) was super special grade (Lot. s3502) from Seikagaku Kogyo Co., Ltd.. The characteristic properties of the ionic polysaccharides used are listed in Table I.

Aqueous solutions of these ionic polysaccharides were passed through a mixed-bed ion-exchange resin column (Amberlite IR-120 and IRA-400) and the acid form polymers thus obtained were completely neutralized with sodium hydroxide in a potentiometric titration. For pH measurements, a Horiba Model F-7ss pH meter sensitive to 0.002 pH unit was used in conjunction with a Horiba 6026 combination electrode. The polyelectrolyte concentration was calculated from the potentiometric titration data. The polysaccharide solutions thus prepared were diluted to the desired concentration with double-distilled water. All other chemicals used were reagent grade.

Preparation of BPA Monomer. The dimer and higher aggregates usually present in BPA samples have been known to have a pronounced effect on the rate of heat denaturation of BPA (Petersen and Foster, 1965a) as well as on the pH–solubility profile (Petersen and Foster, 1965b; McMenamy and Lee, 1967). Thus the fractionation of BPA is indispensable to the study of heat denaturation. The dimer and higher aggregates were separated from monomer by Sephadex G-150 exclusion chromatography following essentially the procedure described by Pedersen (1962). The monomer fraction of BPA, which exhibited a unique monomer band in polyacrylamide electrophoresis and a single symmetric peak (4.3 S) in the analytical ultracentrifuge, was subjected to exhaustive dialysis against double-distilled water at 4 °C. The protein was subsequently deionized by passage through a mixed-bed ion-exchange column (Amberlite IR-120 and IRA-400) to get isoionic protein. BPA solutions thus obtained were concentrated to the desired concentration using a Diaflo Ultrafilter PM10 (Amicon) and the concentrated BPA solution was stored at 4 °C as a stock solution of BPA monomer. The concentrated BPA solution showed a very faint dimer band in addition to the main monomer band in disc electrophoresis which was invariant over the period of all experiments. The rate of heat denaturation of this monomer BPA at 65 °C was approximately three times slower than that of nonfractionated BPA. Comparison with the data of dimer content dependence on the rate of heat denaturation of BPA by Petersen and Foster (1965a) suggests that the dimer content in stock BPA solution is negligibly small.

Heat Denaturation. A mixture of 4 mL of phosphate buffer (pH 5.39, ionic strength 0.106) and 0.5 mL of ionic polysaccharide solution in a 10 × 120 mm test tube with a silicone rubber stopper was preheated in a thermostat controlled at a temperature a few degrees higher than the desired denaturation temperature. The protein solution thermally equilibrated at 32 °C (0.5 mL) was added to the above buffer–polysaccharide mixture and immediately mixed by agitation, and the test tube was immersed in a thermostat bath at a controlled denaturation temperature (60, 65, and 70 °C). These procedures were performed as quickly as possible (within a few seconds), so that the mixture was practically at a denaturation temperature at the starting time. At the end of the desired heating time, the test tube was removed and quickly cooled in an ice bath at about −10 °C to stop denaturation as rapidly as possible. In order to avoid the additional denaturation which may occur at low temperature, the test tube was kept in the ice bath for 30 s till the temperature of sample was cooled down to room temperature, then the test tube was removed from the bath to keep it at room temperature. The heating and cooling conditions used were confirmed to be satisfactory for the kinetic study of heat denaturation by preliminary experiments. The reaction mixture was centrifuged at room temperature for 20 min at 20000 rpm (43000*g*) to remove the precipitate present by using a Hitachi 20 PR centrifuge. The concentration of residual protein in the resulting supernatant was determined by the micro-Kjeldahl method on the basis of 16.1% nitrogen/g and by ultraviolet absorption at 278 nm using a Hitachi 124 spectrophotometer. The extinction coefficient of BPA was assumed to be 6.67 dL/(g cm). The protein concentration measured by both methods coincided within experimental error.

RESULTS AND DISCUSSION

Effect of Polysaccharide Concentration. The effect of polysaccharide concentration on protein denaturation was pursued by measuring the turbidity of the bulk solution and the amount of heat-precipitated protein at room temperature. As shown in Figure 1a, the turbidity for the heated protein solutions in the presence of Cm-dextran and

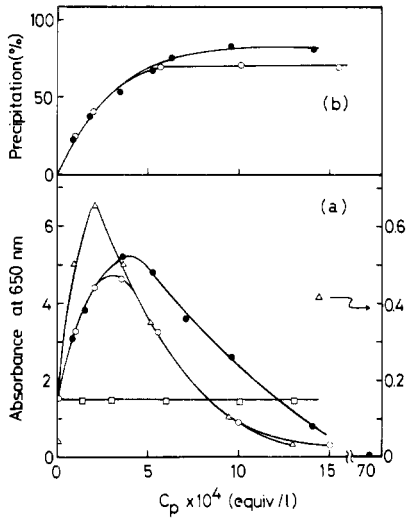

Figure 1. Effect of polyelectrolyte concentration, C_p, on heat precipitation of BPA: (a) the relation between polyelectrolyte concentration and turbidity; (b) the relation between polyelectrolyte concentration and amount of precipitated BPA. Protein concentration, 0.342%. Added polysaccharides and heating time: (●) CMD-11, 5 min at 70 °C; (○) DS-3, 5 min at 70 °C; (△) CMD-11, 30 min at 60 °C; (□) CMD-6, 5 min at 70 °C.

dextran sulfate with degree of substitution 2.54 (CMD-11 and DS-3) increases with increasing polysaccharide concentration (C_p) in the low C_p region. After reaching a maximum C_p value around 0.0002 to 0.0004 equiv/L, it decreases slowly with increasing C_p and finally disappears at high polysaccharide concentration. This result shows a possibility that the addition of these dextran derivatives may protect the proteins from the heat precipitation. The polysaccharide concentration corresponding to the maximum turbidity depends on the denaturation temperature and the kind of ionizable groups of the added polysaccharide: the number of ionizable groups of polysaccharide per molecule of BPA at the maximum turbidity is calculated to be approximately 4 and 8 for Cm-dextran at 60 and 70 °C, respectively, and to be 6 for dextran sulfate at 70 °C. It is obvious that these turbidity changes are not brought about from only the salting in or out effect by added polyions since the addition of NaCl or phosphate ion corresponding to such small ionic strength change due to the polyions did not cause any measurable turbidity change. The result that an addition of dextran had no effect on the turbidity behavior, on the other hand, suggests that the effect of dipole–dipole or dipole–ion interaction between the OH groups of dextran and the protein may be regarded as negligible for heat precipitation of BPA as well as the excluded volume effect by the dextran molecule. Thus, the observed turbidity change can be regarded as resulting from mainly the electrostatic interaction between BPA and these polyions.

Figure 1b shows that the amount of heat-precipitated protein increases with increasing C_p of Cm-dextran or dextran sulfate in a low C_p region and becomes saturated at about twice the concentration corresponding to the maximum turbidity. On the other hand, the supernatant gave the following observations in polyacrylamide disc electrophoresis and circular dichroism spectra: (1) electrophoresis of supernatant shows a clear characteristic monomer band only for the C_p region lower than 0.0005 equiv/L, but shows a few bands such as dimer and trimer in addition to monomer for the higher C_p region; and (2) circular dichroism spectra of the residual protein in the supernatant in the low C_p region are close to those of native BPA, but for the residual protein in the solution containing

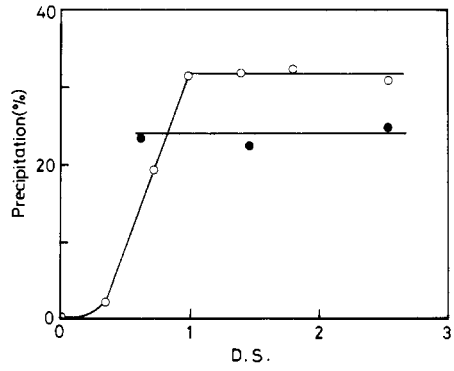

Figure 2. Effect of degree of substitution (D.S.) of Cm-dextran (○) and dextran sulfate (●) on heat precipitation of BPA: protein concentration, 0.342%; polyelectrolyte concentration, 0.0002 equiv/L; heating time, 5 min at 65 °C.

much polysaccharide the molecular ellipticity is remarkably decreased and the peak at 222 nm disappears. These observations suggest that the heat denaturation of BPA is promoted by added polysaccharide and that the heat-denatured protein is completely precipitated by centrifugation in the low C_p region and the heat-denatured protein remains soluble in the supernatant in the high C_p region. Therefore, it is advantageous to select a proper low C_p in order to estimate the degree of heat denaturation through the amount of heat precipitated protein. In the following studies, we adopted a fixed condition, 0.0002 equiv/L as the polyelectrolyte concentration and 0.342% as the protein concentration, unless stated otherwise.

Effect of Ionic or Hydrophobic Group Density. In order to clarify the effect of the charge density of polysaccharides on heat denaturation of BPA, we measured the fraction of heat precipitated BPA in the protein solutions containing Cm-dextran or dextran sulfate with various amounts of substituted ionizable group. If the promotion of heat precipitation of BPA by Cm-dextran is attributed to the electrostatic interaction only, the fraction of precipitated protein should increase with increasing degree of substitution of carboxyl group since the electrostatic free energy and the amount of hydration of Cm-dextran increase monotonously with increasing degree of substitution (Gekko and Noguchi, 1975, 1974). As shown in Figure 2, however, the fraction of precipitated protein increased abruptly with increasing degree of substitution of the carboxyl group for Cm-dextran with degree of substitution less than 1, but it leveled off for degree of substitution higher than 1. In the case of dextran sulfate, similarly, the fraction of precipitated BPA was independent of degree of substitution higher than 0.6. Unfortunately we could not obtain the data in the low degree of substitution region, but we can expect an existence of a critical degree of substitution less than 0.6, which is smaller than that for Cm-dextran. This result coincides qualitatively with data obtained by Bernfeld and Kelley (1963) that other sulfated polysaccharides which had at least 0.6 sulfate groups per repeating unit are potent inhibitors of lipoprotein lipase. At present, any model on ionic polysaccharide–protein interaction does not appear to explain reasonably the existence of this critical degree of substitution. It may be necessary to take into consideration some additional factor such as geometrical or steric structure in the electrostatic interaction between polyion and protein.

It is interesting to clarify the effect of hydrophobic groups bound to polysaccharide on protein stability as compared to the substituted ionic groups in consideration of future utilization of such polysaccharides. We have measured the fraction of protein precipitated by heating

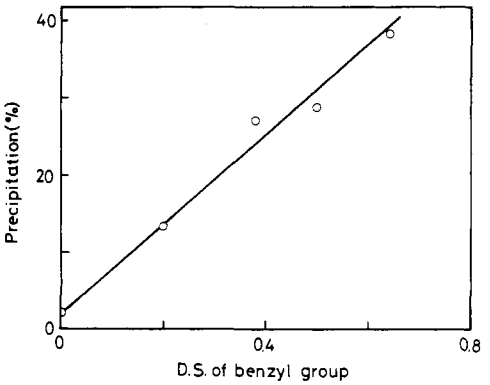

Figure 3. Effect of hydrophobic density of Cm-Bzl-dextran on heat precipitation of BPA: heating time, 5 min at 65 °C; protein concentration, 0.342%; polyelectrolyte concentration, 0.0002 equiv/L.

in the presence of Cm-Bzl-dextran having various degrees of substituted benzyl group and constant degree of substitution of carboxyl group 0.35. As shown in Figure 3, the fraction of precipitated protein after heating for 5 min at 65 °C increased in proportion to the increase in the content of benzyl group, following the equation:

$$\text{fraction of precipitation (\%)} = 56.5 \times \text{degree of substitution of benzyl group} + 2.6 \quad (1)$$

This proportionality suggests that the hydrophobic interaction of Cm-Bzl-dextran with BPA is effective. On the other hand, it is seen that the fraction of precipitated protein by Cm-Bzl-dextran having degree of substitution of benzyl group 0.64 and degree of substitution of carboxyl group 0.35 is larger by approximately 20% than that of Cm-dextran having degree of substitution of carboxyl group 0.99, which corresponds to Cm-dextran having degree of substitution of carboxyl group 0.35 and an additional degree of substitution of carboxyl group 0.64. Then we can conclude that the heat precipitation of BPA is more strongly affected by introducing a hydrophobic benzyl group than by introducing an ionic carboxyl group into dextran.

Effect of Degree of Polymerization. In order to clarify the effect of degree of polymerization or molecular weight of added polysaccharide on heat precipitation of protein, we measured the precipitation of BPA by heating in the presence of Cm-dextran with various degrees of polymerization $\overline{DP}$ (sample No. CMD-1, -2, -3, -4, -5, and -9). The small difference in degree of substitution of carboxyl group (approximately 1.5) of Cm-dextran used can be neglected in the discussion since there is no dependence of degree of substitution of carboxyl group on heat precipitation of BPA in the region of degree of substitution larger than 1 as seen in Figure 2. As shown in Figure 4, the fraction of the protein precipitated by heating is almost independent of $\overline{DP}$ for Cm-dextran of $\overline{DP} > 37$, but an abrupt decrease of precipitation is observed for Cm-dextran of $\overline{DP} = 7.7$. It is probable that a critical $\overline{DP}$ in the effect of Cm-dextran on heat precipitation of BPA exists at some $\overline{DP}$ between 12 and 37, considering that a conformational transition from random coil to rodlike form occurs at around $\overline{DP} = 12$ (Gekko and Noguchi, 1971; Gekko, 1971a, b). Cm-dextran of low $\overline{DP}$ may not interact with protein so strongly as to cause a large change of charge distribution on the surface of the protein because low molecular weight Cm-dextran can not produce a condensed electrostatic field as well as low-molecular-weight electrolytes such as sodium acetate.

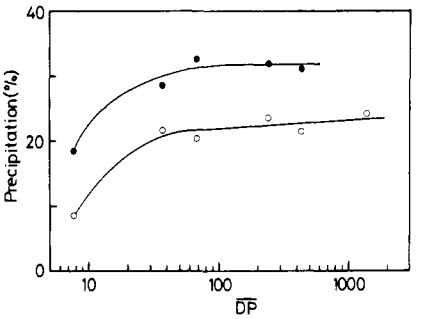

Figure 4. Effect of degree of polymerization ($\overline{DP}$) of Cm-dextran on heat precipitation of BPA: protein concentration, 0.342%; polyelectrolyte concentration, 0.0002 equiv/L; heating time, 5 min at 65 °C (○) and 30 s at 70 °C (●).

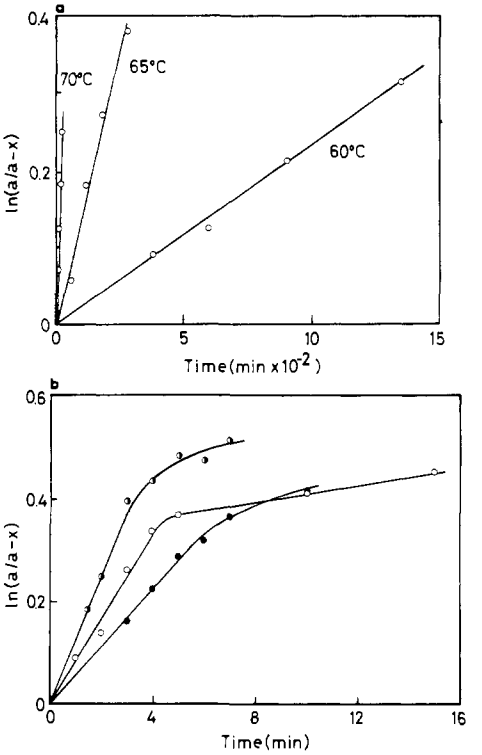

Figure 5. (a) Plots of ln $(a/a - x)$ against time (t) for heat precipitation of BPA; (b) typical plots of ln $(a/a - x)$ against time at 65 °C for heat precipitation of BPA in the presence of DS-3 (●), CMD-11 (○), and CMBD-4 (◑): protein concentration, 0.342%; polyelectrolyte concentration, 0.0002 equiv/L.

Kinetic Studies of Heat Precipitation. The kinetic study of heat denaturation of BPA, which is very rare because of the complexity of the behavior, has been performed by using a solubility method (Levy and Warner, 1954; Warner and Levy, 1958). If the rate of heat precipitation of protein observed under a fixed condition is proportional to the rate of heat denaturation, it should be a first-order reaction with respect to protein concentration since the essential step in denaturation is a unimolecular structure change such as unfolding or isomerization. Therefore, the following kinetic equation for a first-order reaction should be satisfied

$$k = \frac{1}{t}\left[\ln\left(\frac{a}{a-x}\right)\right] \quad (2)$$

where k is the rate constant of the first-order reaction, a the initial concentration of protein at time 0, and x the concentration of precipitated protein at any subsequent time t. In this work, we estimated the rate of heat precipitation of BPA in the presence or absence of ionic dextran derivatives to know their effects on kinetic pa-

Table II. Rate Constant of Heat Precipitation of BPA (pH 5.39, Ionic Strength 0.085, Protein Concentration 0.342%)

added polymer	C_p, equiv/L	k, s^{-1} 60 °C	65 °C	70 °C
none	0	$3.88 \cdot 10^{-6}$	$2.54 \cdot 10^{-5}$	$2.02 \cdot 10^{-4}$
CMD-11	0.0002	$3.22 \cdot 10^{-4}$	$1.42 \cdot 10^{-3}$	$8.73 \cdot 10^{-3}$
DS-3	0.0002	$1.06 \cdot 10^{-4}$	$9.12 \cdot 10^{-4}$	$6.74 \cdot 10^{-3}$
CMBD-4	0.0002	$3.89 \cdot 10^{-4}$	$2.08 \cdot 10^{-3}$	$7.98 \cdot 10^{-3}$
CS-A	0.0002		$1.15 \cdot 10^{-3}$	

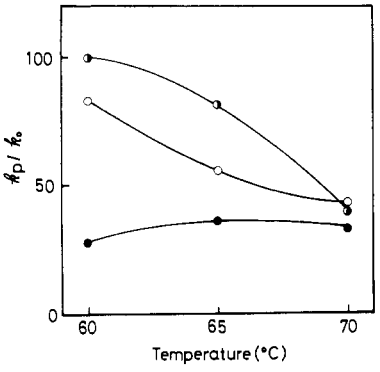

Figure 6. The ratio of rate constants for heat precipitation of BPA in the presence of polysaccharides (k_p) to rate constant in the absence of them (k_o): (●) DS-3, (○) CMD-11, and (◑) CMBD-4.

rameters. The plots of ln $(a/a - x)$ vs. t for heat precipitation of BPA in buffer are shown in Figure 5a. The typical plots of ln $(a/a - x)$ vs. t for heat precipitation at 65 °C for the systems containing three kinds of polysaccharides are shown in Figure 5b. The similar graphs were obtained at temperatures of 60 and 70 °C. There was a proportionality at the first stage between the values of ln $(a/a - x)$ and t, and a lag period was not observed at any denaturation temperature. This result satisfies the requirement that this reaction is a first-order reaction in the initial stages. However, the rates for heat precipitation decreased with time and deviated from the first-order plot in the latter stages, suggesting that additional factors may affect the reaction kinetics. Judging from the results presented in Figure 1a, this departure from linearity would be expected if the ratios of polysaccharide to available protein considerably increases, which might occur when the soluble protein diminishes with denaturation. In this work, the values of rate constant, k, were estimated by using the least-square method for the linear parts at the initial stages where this ratio of polysaccharide to available protein would not be substantially affected by denaturation. The results are summarized in Table II. The rate constant for the systems containing polysaccharides is very large compared with that for BPA alone at any temperature. That is, the heat precipitation of BPA is remarkably accelerated by adding ionic polysaccharides. The ratio of rate constants for the systems with and without added polysaccharide, k_p/k_o, which may be a measure of acceleration, is plotted against temperature in Figure 6, where k_p and k_o are the rate constants in the presence and absence of polysaccharide, respectively. Addition of Cm-dextran or Cm-Bzl-dextran causes a larger acceleration of heat precipitation which decreases with increasing temperature. The large acceleration by Cm-Bzl-dextran may be attributed mainly to the hydrophobicity of this polymer, since the amount of substituted carboxyl group in this polymer is not so large as to cause the remarkable acceleration of heat precipitation. In the case of dextran sulfate, the ratio is almost independent of temperature.

Table III. Thermodynamic Parameters for Heat Precipitation of BPA at 65 °C

added polymer	C_p, equiv/L	$\Delta G^{\ddagger}$, kcal/mol	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, cal/deg·mol
none	0	27.0	83.3	167
CMD-11	0.0002	24.3	65.6	122
DS-3	0.0002	24.6	89.1	191
CMBD-4	0.0002	24.1	61.3	110

This result suggests that there is a specific interaction of BPA with dextran sulfate different from the interaction with Cm-dextran. The rate constant for chondroitin sulfate is between that of dextran sulfate and Cm-dextran as expected from the structure of chondroitin sulfate, which contains the same amount of carboxyl group and sulfate group per repeating unit.

The thermodynamic parameters, $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$, for these precipitation reaction are listed in Table III. These thermodynamic parameters were calculated by the following equations for the critical activation state along the pathway from the native state to denatured state of protein

$$k = A \exp\left(\frac{-\Delta E}{RT}\right) \tag{3}$$

$$K = \frac{KT}{h} \exp\left(\frac{-\Delta G^{\ddagger}}{RT}\right) = \frac{KT}{h} \exp\left(\frac{-\Delta H^{\ddagger}}{RT}\right) \exp\left(\frac{\Delta S^{\ddagger}}{R}\right) \tag{4}$$

$$\Delta H^{\ddagger} = \Delta E - RT \tag{5}$$

where A is the frequency factor; ΔE, activation energy; T, absolute temperature; R, gas constant; K, Boltzmann constant; h, Planck constant; $\Delta G^{\ddagger}$, free energy of activation; $\Delta H^{\ddagger}$, enthalpy of activation; and $\Delta S^{\ddagger}$, entropy of activation. The value of $\Delta G^{\ddagger}$ in the presence of ionic polysaccharides was found to be smaller by 2–3 kcal/mol than that of the reference system without them. It is noteworthy that the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ change to the same direction (increase or decrease) together by adding ionic polysaccharides and that both values are decreased by addition of Cm-dextran and Cm-Bzl-dextran, but conversely they are increased by addition of dextran sulfate. This means that heat precipitation of BPA in the presence of these polysaccharides is the compensation reaction of the enthalpy change and the entropy change of activation so as to make the free-energy change of activation small, and that the acceleration of heat precipitation by Cm-dextran or Cm-Bzl-dextran is induced by the enthalpy change overcoming the entropy change while the acceleration by dextran sulfate is mainly due to the large increase in the entropy of activation. It is interesting to discuss a distinguishable difference in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ with respect to the constitution of added polysaccharides. At present, however, there is no quantitative analysis of these activation parameters since the definite structure of activated protein remains unclarified. It has been supposed that the activated state of protein is close to the structure which native protein is swollen to absorb some content of solvent inside of the molecule by its subtle conformational change (Lumry and Biltonen, 1969). If this is the case, it can be expected that such subtle conformational change or swelling state of protein is sensitively influenced by the interaction of different mode with polysaccharides.

Some interesting results have been reported in relation to the interaction of BPA with ionic polysaccharides (Gorter and Nanninga, 1953; Bettelheim et al., 1966; Noguchi, 1956, 1960). Noguchi (1960) found that car-

boxymethylcellulose and dextran sulfate form a soluble complex with BPA in the pH range of 5.2–5.6 and that the SO_3^- groups on polyions have a stronger affinity to BPA than COO^- groups. This specificity among both ionizable groups for the interaction with BPA was explained by proposing that SO_3^- groups have a stronger tendency to form hydrogen bonds with amino groups on proteins than with COO^- groups. As our experimental condition is very close to the reported one, such a soluble complex should be formed between added dextran derivatives and BPA. The existence of hydrogen bonds is suggested also by our observation that the mixed solution of concentrated dextran sulfate and BPA does not produce a precipitation or turbidity at room temperature but produces a faint turbidity at 4 °C, which is thermally reversible. Such a change in turbidity was not observed for the mixture of Cm-dextran or dextran and BPA. It is probable that Cm-dextran interacts with BPA by the electrostatic force between COO^- groups on the polysaccharide and locally distributed positive charges on the protein as has been proposed (Klotz and Ayers, 1953; Tsong and Thompson, 1965) and that such interaction results in effective changes in charge distribution on the protein surface to facilitate the expansion or partial unfolding of the peptide chain. Thus, the decrease in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ with Cm-dextran may arise mainly from an electrostatic contribution. In the case of dextran sulfate, the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ increased rather slightly, although both parameters should decrease as well as in the case of Cm-dextran if dextran sulfate interacts with BPA through only an electrostatic force. It is speculative that some additional force between dextran sulfate and BPA, probably a hydrogen bond as mentioned above, would affect the activation state accompanying the increase in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ enough to compensate their decrease arising from electrostatic contribution. The result on Cm-Bzl-dextran suggests that a hydrophobic interaction between polymer and protein may cause the decrease in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$.

There is naturally a limitation in a quantitative interpretation of the thermodynamic parameters obtained because the heat denaturation or aggregation of BPA is influenced not only by interaction with added polysaccharide but also by disulfide interchange (Levy and Warner, 1954; Warner and Levy, 1958), heterogeneity in the secondary and tertiary structure (Štokrová and Šponar, 1963), and dimer content (Petersen and Foster, 1965a). Nevertheless, the results obtained in this work strongly prove that both the stability and solubility of protein on heating are dominantly affected by the characteristic interaction between polysaccharide and protein depending on the constitution of added polysaccharides. Further accumulation of thermodynamic datum for these complicated systems, which is very rare at present, should be desired to understand more clearly the role of polysaccharide on heat precipitation of protein. The fact that the added ionic polysaccharide can prevent the proteins from heat precipitation while it may promote heat denaturation may be applicable in food processing.

ACKNOWLEDGMENT

The authors would like to thank Neil Tweedy for reading the manuscript.

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Received for review January 30, 1978. Accepted August 2, 1978. This work was supported by Grant 943017 from the Ministry of Education in Japan.