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Effect of sucrose on the thermodynamic incompatibility of different biopolymers

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The influence of sucrose on the thermodynamic incompatibility of a number of biopolymers in aqueous solutions has been studied. Three pairs of the biopolymers were chosen: sodium caseinate–ovalbumin, 11S globulin from vicia faba–ovalbumin and sodium caseinate–sodium alginate.

The cosolubility of the biopolymers was investigated at different sucrose concentrations in solution (in the range 0–50% w/v). A big increase in the cosolubility of the biopolymers was observed with increasing sucrose concentration. It was established that the increasing cosolubility of the biopolymers occurs in accordance with the increase in the protein solubility in the aqueous medium on sucrose addition. It was supposed that the same factor provides the basis of both the increase in the solubility of proteins and the cosolubility of the biopolymers in the aqueous medium. The thermodynamic parameters of the different pair interactions (the second virial coefficients) were estimated using light scattering data in the binary and ternary aqueous solutions of the biopolymers without sucrose and on the addition of 25% w/v of sucrose.

INTRODUCTION

Thermodynamic incompatibility is one of the most commonly encountered phenomenon in mixed biopolymer solutions (Tolstoguzov, 1986, 1992. Semenova *et al.*, 1990, 1991a). This phenomenon can be a controlling factor on the structure and physico-chemical properties of food (Tsapkina *et al.*, 1992; Dickinson & Semenova, 1992; Pavlovskaya *et al.*, 1993). The nature and degree of thermodynamic incompatibility of the biopolymers depends on the interactions between all the components in the solution (Tolstoguzov, 1992; Semenova *et al.*, 1990, 1991a). Altering the composition of the aqueous medium by adding different low molecular weight substances can influence the thermodynamic incompatibility of biopolymers. The effect on biopolymer thermodynamic incompatibility of low molecular weight components such as inorganic salts and acids/alkalis (the role of pH) has been quite extensively studied (Pavlovskaya *et al.*, 1993; Semenova *et al.*, 1991a; Antonov *et al.*, 1979; Polyakov *et al.*, 1979).

Sucrose is the second (after NaCl) most abundant low molecular food taste additive, but the role of sucrose on the thermodynamic incompatibility of biopolymers in aqueous media has not been studied. Sucrose is the dominant mass component in a wide variety of food. For instance, the sucrose content in ice cream can be as high as 33% w/w and 60% w/w in the concentrates of some drinks.

In this connection, we have attempted to study the influence of sucrose on the thermodynamic incompatibility of a number of the biopolymers in an aqueous medium. Three pairs of biopolymers were chosen as the object of our investigations, namely, caseinate–ovalbumin, 11S globulin from vicia faba–ovalbumin and caseinate–alginate. In deciding on the biopolymer combinations we were guided both by scientific interest and manufacture practice. For example, caseinate, ovalbumin and alginate are widely used in ice cream manufacture, and plant legume proteins (in our study it was the 11S globulin from vicia faba) are one of the promising materials for formulating new forms of food products (Fauconneau, 1983; Belitz & Grosch, 1987).

The preliminary results of this investigation have been presented by Tolstoguzov (Tolstoguzov, 1992).

EXPERIMENTAL

Materials

11S globulin from broad beans (vicia faba)

A sample of lyophilized legumin was obtained as described elsewhere (Popello *et al.*, 1988). Ninety-five percent of the sample had a 11S sedimentation

coefficient, the remaining 5% had a sedimentation coefficient of 15S.

Alginate

Sodium alginate was purchased from Fluka Chemicals. The sample was reprecipitated by acidic isopropanol as reported by Plashchina *et al.* (1985).

Ovalbumin and caseinate

Lyophilized ovalbumin (recrystallized five times) and caseinate (high purity grade) were purchased from Russian sources.

Sucrose and other chemicals

A high purity grade sucrose was employed. The Ca^{2+} content in the sucrose was less than 0.05% w/w.

Double distilled water was used for solution preparation. For buffer preparation, analytical grade Na_2HPO_4 and NaH_2PO_4 were used.

METHODS

Determination of solubility and cosolubility of biopolymers

The biopolymers were dissolved in water at pH 8 and titrated to a predetermined pH. The resulting solutions were divided into two and the required quantities of sucrose were added to one of them. Thereafter the solutions were centrifuged. The same procedure was extended to biopolymer mixtures with predetermined concentrations of constituents. The operating conditions for centrifugation were 30 min. at 4000 *g* for 11S globulin and ovalbumin and 1 h at 35,000 *g* for caseinate as well as for the biopolymer mixtures. The supernatant concentrations of the individual biopolymers were evaluated refractometrically and in the case of the proteins by the biuret method (Gernall *et al.*, 1949). For the biopolymer mixtures, the total biopolymer concentration of the upper phase was determinated refractometrically.

A Shimadzu differential refractometer (Japan) at 436 nm wavelength was used.

Within the limits of experimental error, the refractive index increments of the biopolymers remained unchanged in the presence of sucrose.

The refractive index increments in 0.1 mol/dm^3, pH 7.0 were: 11S globulin and caseinate — $(0.2\pm0.003)*10^{-6}$ m^{3}kg^{-1}, ovalbumin — $(0.183\pm0.003)*10^{-6}$ m^{3}kg^{-1}, and alginate — $(0.15\pm0.003)*10^{-6}$ m^{3}kg^{-1}.

Light scattering

Biopolymers were dissolved in aqueous phosphate buffer solutions (0.1 mol/dm^3, pH 7.0) to obtain stock solutions. A set of solutions at lower concentrations were prepared by dilution of these solutions with buffer.

The light scattering experiments were carried out using nephelometer FPS-3 (Unique Design Bureau of Scientific Instruments, Academy of Science, USSR) with non-polarized light at a wavelength of 436 nm. Since the legumin and ovalbumin solutions as well as their mixture did not exhibit an angular dependence of light scattering they were investigated at a fixed scattering angle of 90°. For caseinate and alginate and mixtures containing these biopolymers, measurements were made at scattering angles in the range 45 140°. The instrument was calibrated with dust free benzene (R_{90} = $47.4*10^{-2}$ m^{-1}). Prior to the experiment, all solutions were filtered through membranes with a pore size of 0.45 μm (Sartorius) directly into the light scattering cells.

In the case of 11S globulin, ovalbumin and their mixtures, the results were used to plot the concentration dependence of the ratio $(HC/(\Delta R_{90}))$ for binary solutions and the ratio $(H'(C_2 + C_3)/\Delta R_{90})$ for ternary solutions, where H and H' are optical constants of the systems: $H = 2\pi^2n^2v^2/N_A\lambda^4$, $H' = 2\pi^2n^2v^2/N_A\lambda^4$; C is the concentration of the biopolymers; ΔR_{90}, excess light scattering of biopolymer solutions over the solvent. For the three-component systems, the slope of the concentration dependence of the ratio $(H' = (C_2 + C_3)/\Delta R_{90})$ can be expressed as follows (Kratochvil & Sudelof, 1986):

$$tg\alpha = 2\frac{v_2^2M^2_{2w}w_2^2A_{22} + 2v_2v_3M_{2w}M_{3w}w_2w_3A_{23} + v_3^2M^2_{3w}w_3^2A_{33}}{(v_2^2M_{2w}w_2 + v_3^2M_{3w}w_3)^2} \quad (1)$$

where n is the refractive index of the solvent; N_A, is Avogadro's number; λ is the wavelength of incident light in vacuum; A_{22} and A_{33} are the second virial coefficients characterizing the interaction of polymer 2_x-polymer 2 and polymer 3-polymer 3, respectively; A_{23} is the cross second virial coefficient characterizing the interaction of polymers 2 and 3 with each other; v_2 and v_3 are the refractive index increments of polymers 2 and 3; w_2 and w_3 are the weight fractions of polymers 2 and 3; M_{2w} and M_{3w} are the weight average molecular weight of polymers 2 and 3; C_2 and C_3 are the concentrations of polymers 2 and 3; ΔR_{90} is the excess light scattering at a 90° angle.

In the case of alginate, the experimental data were analyzed by the use of the double extrapolation method of Zimm (1948).

To handle experimental data for caseinate and biopolymer mixtures containing caseinate, the Yang method was used (Yang, 1957).

The experimental error in the determination of molecular weight and the second virial coefficient of the biopolymers in the binary solutions were not greater

than 10%. For the cross second virial coefficient the maximum error was 30%.

RESULTS AND DISCUSSION

The thermodynamic incompatibility of biopolymers and phase separation were observed for all ternary biopolymer solutions under investigation. A large decrease in the lower phase volumes and a corresponding large increase in the upper phase volume was observed in the presence of the high sucrose concentration in the system. An increase in the biopolymer concentration in the upper phase was also observed. On the basis of these data, evidently, it is possible to suppose that the cosolubility of the biopolymers in the upper phase increases at high sucrose concentrations.

Figure 1 shows the increase in the total biopolymer concentration in the upper phase of the mixed biopolymer solution in presence of the sucrose in the aqueous medium. The most significant rise in the biopolymer concentration is observed up to 25% w/v of sucrose in the solutions. A further increase in the sucrose concentration in aqueous medium up to 50% w/v does not lead to a change in the cosolubility of the biopolymers as, for example, is observed for a mixture of the two different proteins or causes only a slight increase in the biopolymer cosolubility as is the case for mixtures of caseinate and alginate.

Figure 2 displays changes in the solubility of the proteins under investigation with increasing sucrose concentration in aqueous medium (from 0 to 50% w/v). In the case of the caseinate, a significant increase in the influence of sucrose on protein solubility is observed on decreasing the pH towards the protein isoelectric point (IEP) (from 7.0 to 5.1).

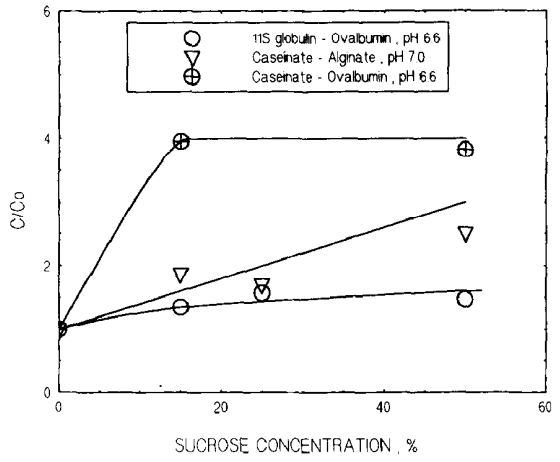

Fig. 1. Influence of sucrose concentration on the cosolubility of different pairs of biopolymers in aqueous medium. C and C_0 are the total biopolymer concentrations, respectively, with and without sucrose.

Alginate completely dissolves in the aqueous medium under the experimental conditions employed. On comparing Figs 1 and 2, it can be observed that the cosolubility of the biopolymers change with sucrose in a similar way. It therefore appears that an increase in protein solubility and biopolymer cosolubility in the presence of sucrose is governed by similar mechanisms.

In order to elucidate the influence of the sucrose on molecular parameters of the biopolymers and on the thermodynamic parameters of their pair interactions in aqueous medium, the systems were studied by light scattering.

Figures 3a and b shows the light scattering data for 11S globulin and ovalbumin, with and without 25% w/v sucrose.

Figure 4 exhibits the concentration dependences of

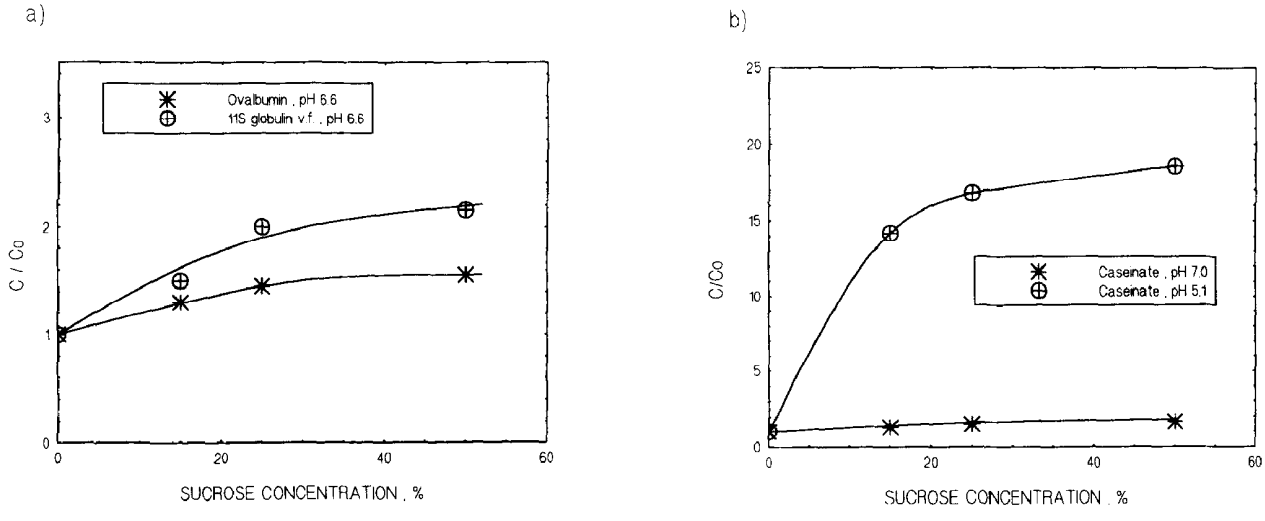

Fig. 2. Influence of sucrose concentration on the solubility of different biopolymers in aqueous medium. C and C_0 are the biopolymer concentrations, respectively, with and without sucrose.

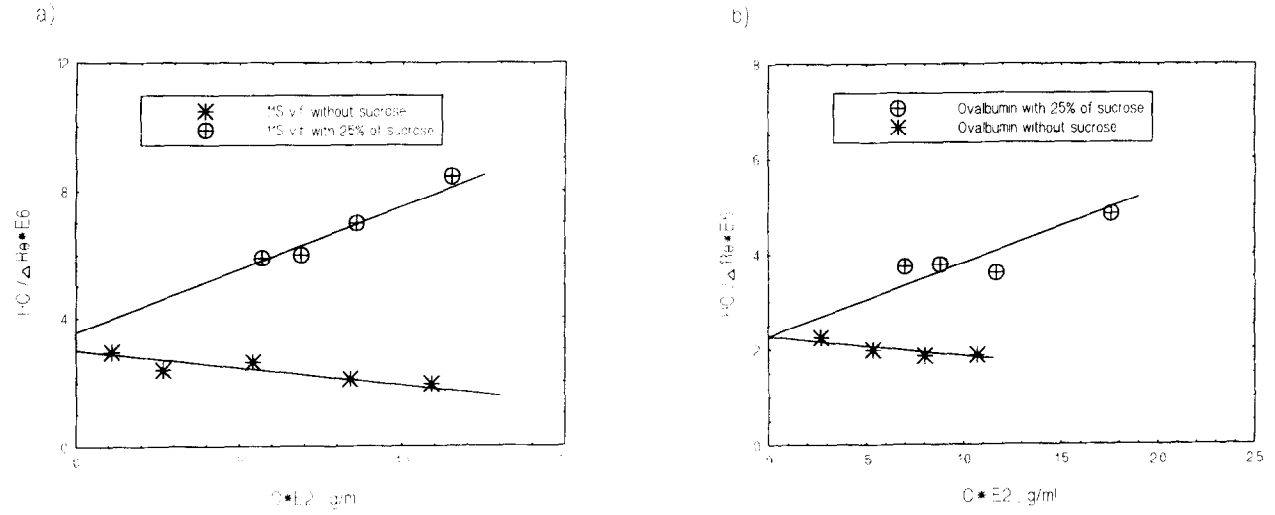

Fig. 3. The excess light scattering of the proteins in aqueous solution with and without 25% sucrose (0.1 mol/dm^3 phosphate buffer, pH 7.0): (a) 11S globulin vicia faba; (b) ovalbumin.

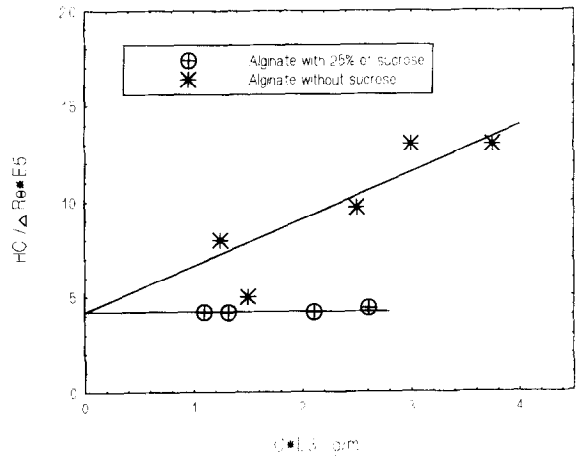

Fig. 4. The excess light scattering of alginate in aqueous solution with and without 25% sucrose (0.1 mol/dm^3 phosphate buffer, pH 7.0).

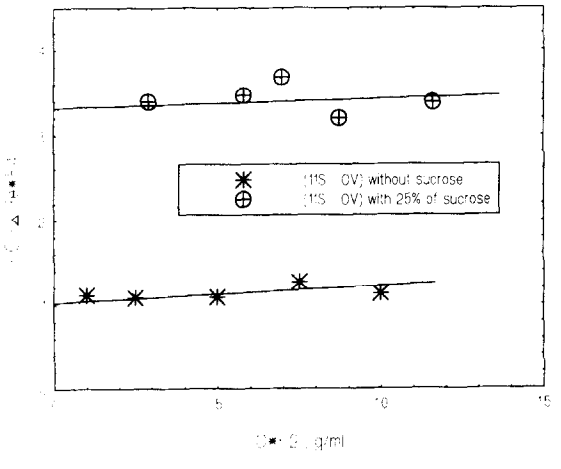

Fig. 5. The excess light scattering of 11S globulin vicia faba–ovalbumin mixture in aqueous solution with and without 25% sucrose (0.1 mol/dm^3 phosphate buffer, pH 7.0).

light scattering for the alginate solutions with and without 25% w/v of sucrose.

Figure 5 presents light scattering data for the equimass mixtures of 11S globulin–ovalbumin with and without 25% w/v sucrose.

Figure 6 shows examples of Yang's extrapolation (Yang, 1957) of light scattering data for caseinate alone and caseinate-containing mixtures.

The numerical results obtained from the light scattering data are given in Tables 1 and 2. Table 1 shows that for the globular proteins (11S globulin and ovalbumin), the presence of 25% w/v of sucrose in solutions causes a significant increase in the protein second virial coefficients. This result shows a change in the quality of the aqueous solvent from a thermodynamically-poor to thermodynamically-good one in the presence of sucrose. It is known that low molecular weight sugars can give rather strong 'polyfunctional' hydrogen bonds with protein molecules in aqueous medium (Jencks, 1969). As a result, protein hydrophilicity increases due to added sucrose molecules which form a hydrophilic layer around the protein molecule; because of this, the hydrophilicity of globular proteins coverge on sucrose addition, giving rise to the thermodynamic compatibility of globular proteins (see Table 2).

Table 1 shows that in the case of the caseinate, hydrogen bond formation between molecules of the sucrose and the protein probably causes disruption of the associates of the caseinate molecules. Caseinate weight average molecular weight decreases at 25% w/v of sucrose in the solution. As this takes place, however, the surface of the new protein associates with lower molecular weight, and appears more hydrophobic ($A_2 < 0$), then the protein associates with higher molecular weight as distinct from globular protein behavior in the presence of sucrose. The ability of the caseinate molecules to form large associates in aqueous medium is well known. In addition

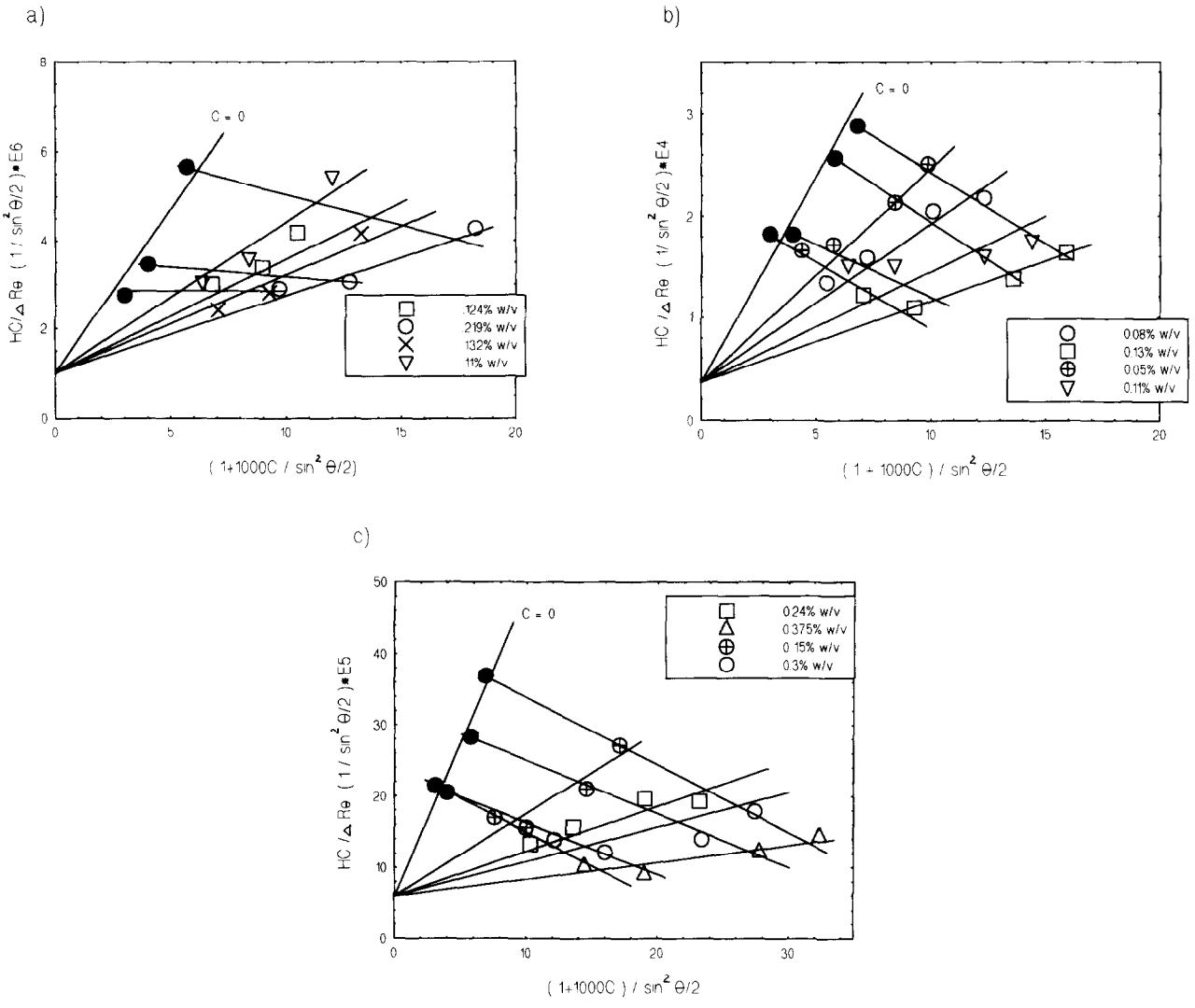

Fig. 6. The excess light scattering of caseinate alone and caseinate-containing mixtures in aqueous solution with 25% sucrose (0.1 mol/dm^3 phosphate buffer, pH 7.0): (a) caseinate; (b) caseinate-alginate; (c) caseinate-ovalbumin.

Table 1. Molecular weight and thermodynamic parameters of the 11S globulin from vicia faba, ovalbumin, caseinate and alginate in aqueous medium with and without 25% w/v sucrose at pH 7.0, $I = 0.1$

Biopolymers	M_w, kDa		$A_2 * 10^4$, m^3 mol kg^{-2}		$A_2 * 10^2$, m^3 mol^{-1}	
	0% w/v sucrose	25% w/v sucrose	0% w/v sucrose	25% w/v sucrose	0% w/v sucrose	25% w/v sucrose
11S globulin	330	280	−0.54	1.98	−1.18	3.11
Ovalbumin	44	44	−1.78	7.6	−0.07	0.29
Caseinate	8600	1400	0.67	−0.45	998	−17.52
Alginate	250	250	12	0	15	0

to calcium ions, the reason for caseinate associate formation may be hydrophobic interactions of the non-polar amino acid residues. (Dyachenko & Vlodavez, 64; Kresheek & Winkler, 1964). Hydrophobic parts of the protein molecules are apparently exposed as a result of the associate decomposition in the presence of sucrose.

For alginate, an anionic polysaccharide with carboxyl groups, sucrose addition leads to the change of the thermodynamic quality of the aqueous medium from thermodynamically good ($A_2 > 0$) to thermodynamically ideal ($A_2 \approx 0$). It seems likely that there is competition for water molecules between sucrose and alginate in the solution; so, alginate hydrophilicity in aqueous medium decreases in presence of high sucrose concentrations.

Table 2 exhibits the effect of sucrose on the

Table 2. Thermodynamic parameters of the pair interactions of the biopolymers in aqueous medium with and without 25% sucrose at pH 7.0, $I = 0.1$

Mixtures	$A_{23} * 10^4$ m^3 mol kg^{-2}		$A_{23} * 10^2$ m^3 mol^{-1}	
	0% w/v sucrose	25% w/v sucrose	0% w/v sucrose	25% w/v sucrose
HS v.f. - Ovalbumin	3.16	−6.37	0.92	−11.77
Caseinate - Ovalbumin	−0.56	−7.47	−4.24	−92
Caseinate - Alginate	1.4	−12.2	60.2	−85.4

interactions between biopolymers in aqueous medium. Interactions between biopolymers become more favorable in the presence of sucrose for all biopolymer pairs under investigation. But the reasons of this sucrose effect, supposedly, differ for different biopolymer pairs; therefore, for globular proteins, it is, probably a rise of protein hydrophilicity in the presence of sucrose. In the case of the mixture of the caseinate with ovalbumin the reason is, apparently, both a decrease in the excluded volume effect because of the reducing caseinate molecular weight and the rise in the ovalbumin hydrophilicity. For the mixture of caseinate with alginate, the main reason, possibly, consists of decreasing the excluded volume effect and reducing the caseinate molecular weight. It is interesting to note that in the case of the mixture of caseinate with ovalbumin the cross second virial coefficient is negative for mixtures both with and without sucrose. This result indicates thermodynamically-favorable interactions between biopolymers which are enhanced when sucrose is present. An estimation of the first derivations of the chemical potentials with respect to concentration in the systems studied indicate that the sufficient conditions for stability of the system with respect to diffusion (Prigozhin & Defey, 1954; Edmond & Ogston, 1968) fail in both cases in accordance with the two-phase state of the solutions of mixtures.

It is interesting to note that the effect of the sucrose on the thermodynamic properties of the biopolymers in aqueous medium differs for biopolymers with different structures. It is possible to observe both an increase in hydrophilicity and hydrophobicity.

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