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Limited Thermodynamic Compatibility of Proteins in Aqueous Solutions

V. I. Polyakov, V. Ya. Grinberg, Yu. A. Antonov and V. B. Tolstoguzov

Institute of Organoelement Compounds, USSR Academy of Sciences, Vavilov St. 28, Moscow V-312, USSR

Summary

Conditions are established for limited thermodynamic compatibility of proteins of different classes, as distinguished by Osborne, in aqueous solutions. The result obtained are in good agreement with the concept known as $\Delta\chi$ - effect.

Introduction

Most of the study on the limited thermodynamic compatibility (LTC) of two polymers in a common solvent has been done with synthetic polymers. Much less study has been concerned with biopolymers, the most notable examples being the studies of the LTC of such biopolymers as proteins and polysacharides in aqueous solutions (ANTONOV et al., 1975, 1977).

This paper presents initial data on the LTC of proteins in aqueous solutions.

Proteins representing different classes distinguished by Osborne includinng albumins (Alb), globulins (Glo), glutelins (Glu) and prolamins (Pro), were studied. Osborne's classification (OSBORNE, 1924) is in fact a thermodynamic one since it is based on the difference in the solubility of proteins in some typical solvents. The essence of classification is given in Table I.

When two proteins are used belonging to two different classes a solvent can be selected which will prove "good" for one protein and "poor" for the other. Under certain conditions (in the case of absolute incompatibility) one of the proteins falls out to form residue while the other remains in the solutions. According to the $\Delta\chi$ - effect theory* (ZEMAN and PATTERSON, 1972; HSU and PRAUSNITZ, 1974), the greater the maximum difference in the intensities of the interaction of polymers with the solvent the

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* $|\Delta\chi| = |\chi_{12} - \chi_{13}|$, where χ_{12} and χ_{13} are used in their ordinary sense.

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more apparent is the LTC. Conditions under which one of

Table I. Thermodynamic classification of proteins*

No	Solvent	Class			
		Alb	Glo	Glu	Pro
I	Water	+	-	-	-
2	Diluted aqueous solution of neutral salt	+	+	-	-
3	Diluted aqueous solution of acid				
	(a) pI**- Δ***	+	+	+	-
	(b) pH<<pI	+	+	+	+
4	Diluted aqueous solution of alkali				
	(a) pI+ Δ	+	+	+	-
	(b) pH>>pI	+	+	+	+
5	Aqueous solution of alcohol	-	-	-	+

the proteins forms the residue and other is in solution correspond to the maximum difference in the intensities of the interaction of proteins with the solvent, i.e. to the maximum of $\Delta\chi$. From absolute incompatibility it is possible to pass to LTC by gradually enhancing the solvent quality with respect to the protein in the residue, the morphology of coexisting phases often also changes. From a two-phase system containing a solid and a liquid phase one can pass to a system which contains two liquid phases. The solvent quality can be controlled by changing pH, the ionic strength of the solution and the temperature.

Experimental

Materials, as described in Table 2.

To facilitate the quantitative estimation of the composition of coexisting phases some of the proteins were preliminary stained with active dyes ("Ciba Geigy) under mild conditions (pH 7, 25 ^{o}C). The content of the dye in stained preparations did not exceed 0.0075 g per 1 g of the protein. The stained preparations did not differ from the initial ones in pI value , solubility and partition coefficients in two-phase systems.

* (+) stands for soluble, and (-) for insoluble.

** pI is isoelectrical point of protein.

*** Δ =2-4 pH units.

Table 2.

Class	Protein	Code	Source
Alb	Bovine serum albumin	BSA	"Chemreagent Works", Olaina, USSR
	Alkaline precursor gelatin	G	-"-
Glo	Fibrinogen	F	-"-
	Soya beans globulins	SBG	Isolated according to ROBBINS et al. (I966)
Glu	Casein according to Hammersten	C	"Chemreagent Works", Olaina, USSR
Pro	Gliadin	Gli	"Serva"

Methods

Ternary solvent(I)-protein(2)-protein(3) systems were prepared by mixing binary I-2 and I-3 solutions carefully purified from insoluble impurities by centrifugation. After mixing the two solutions long enough for the mass exchange to proceed to completion the phase state of the systems was evaluated by the phase-contrast method under the magnification by 380-800 times using the NU-2 microscope ("Carl Zeiss", Jena). When two-phase systems formed, the phases were separated by centrifugation at I65, 000 g during 50 min in the UPR-8 ultracentrifuge ("Biofizpribor", USSR). Protein concentrations in the phases were measured colorimetrically with the UV VIS "Specord" spectrophotometer ('Carl Zeiss", Jena). In some cases total concentrations of proteins in the phases were determined by the dry residue. These analytical results were used to determine the values of the protein partition coefficients for the coexisting phases: $K_i=w_i'/w_i''$, where i=2,3; w_i is the weight concentration of i=th component; the index (') referring to the upper phase and the index (") to the lower one.

Results and Discussion

We first investigated the LTC of proteins of different classes. Preliminary experiments showed that, unlike synthetic polymers, proteins exibit reasonable good compatibility in aqueous solutions. However, on the basis of the $\Delta\chi$-effect concept we have found that if a solvent is "good" with respect to one protein (2) and "poor" with respect to the other (3) all observed systems

containing proteins of different classes demix into two phases (Table 3, rows I-7). The values of protein partition coefficients obtained for phases show that demixing is accompained mainly by the separation of proteins.This is known to be characteristic of the LTC phenomenon.

Experiments intended to study the LTC of proteins of the same class were restricted to the systems of the Alb-Alb and Glo-Glo type (Table 3, rows 8,9). It is characteristic that in this case the LTC can be observed when the solvent is "good' with respect to both proteins. This shows that the traditional conception of the role played by the interaction of macromolecules of differnt types in the LTC phenomenon (SCOTT, I949) may prove to be of importance for protein LTC studies as well.

Table 3. Conditions for the LTC of proteins in aqueous solutions

No	Proteins		r*	pH	C**	t, °C	K_2	K_3
	2	3						
I	BSA	SBG	I2.7/7.5	4.9	0.39	25	2.20	0.I6
2	BSA	C	I5.2/7.7	6.9	0.50	25	2.96	0.IO
3	BSA	Gli	I5.4/7.2	II.o	-	25	7.93	0.36
4	SBG	C	6.I/6.I	6.6	-	25	-	-
5	F	C	7.0/5.8	6.4	0.4I	25	0.44	I.86
6	SBG	Gli	7.3/I2.8	II.0	-	25	3.66	0.II
7	C	Gli	7.8/I3.I	I0.9	-	25	2.75	0.I2
8	BSA	G	7.9/7.9	7.8	I.00	40	0.38	4.08
9	F	SBG	7.0/6.5	7.9	0.40	25	0.7I	I.I6

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* $r=w_2/w_3$ is the ratio of initial weight concentrations of proteins,%/%.

** C is the concentration of neutral salt, mole/l (Nos. I, 5, 8, and 9 for NaCl; No.

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