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ВЗАИМОДЕЙСТВИЕ ПРИРОДНЫХ И СИНТЕТИЧЕСКИХ ПОЛИЭЛЕКТРОЛИТОВ С БЫЧЬИМ СЫВОРОТОЧНЫМ АЛЬБУМИНОМ

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Изучено взаимодействие в водных растворах ряда природных и синтетических полиэлектролитов (ПЭ): хитозана, поли-N,N-диметил-N,N-диаллиламмоний хлорида, натрий карбоксиметилцеллюлозы и ароматического сополиамида, синтезированного на основе дихлорангидрида изофталевой кислоты и двух диаминов 4,4'-(2,2'-дисульфонат натрия)-диаминодифенила и 4,4'-(2,2'-дикарбоновая кислота)-диаминодифенилметила и бычьего сывороточного альбумина (БСА). Показано, что в результате макромолекулярных реакций образуются белок-полиэлектролитные комплексы (БПК), стабилизированные, в основном, электростатическими силами. Для характеристики их состава использовано значение параметра φ, определяемого как количество ионных групп полиэлектролитов в расчете на макромолекулу белка. Методами спектрофотометрии и кондуктометрии установлено, что в исследованных системах при смешивании компонентов при оптимальных условиях проведения реакции образуются комплексы, состав которых соответствует φ ~ 30 – 90. Определены условия существования нерастворимых БПК. В случае участия в реакции белок – полиэлектролит поли-N,N-диметил-N,N-диаллиламмоний хлорида и хитозана максимальный выход продукта наблюдается при pH ≥ 7. Включение во взаимодействие с белком натрий карбоксиметилцеллюлозы и ароматического сополиамида сопровождается смещением диапазона существования БПК в кислую область: максимальный выход продукта наблюдается при pH ≤ 4. Размер образующихся комплексных частиц варьируется в диапазоне от 10 нм ([ПЭ]/[БСА] = 0,01 – 0,05 г/г) до 1,0 – 5,5 мкм ([ПЭ]/[БСА] = 0,1 – 0,35 г/г). В случае относительно короткоцепочечного ароматического сополиамида, содержащего значительное количество неионизованных карбоксильных групп, доля частиц микронного размера составляет около 5 %. Наличие в составе сополиамида как сульфонатных, так и карбоксильных групп открывает дополнительную возможность для регулирования степени превращения в интерполиэлектролитных реакциях, а значит структуры и состава образующихся комплексов.

Ключевые слова: природные и синтетические полиэлектролиты, бычий сывороточный альбумин, интерполиэлектролитные реакции

INTERACTION OF NATURAL AND SYNTHETIC POLYELECTROLYTES WITH BOVINE SERUM ALBUMIN

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Interaction of a number of natural and synthetic polyelectrolytes (PE): chitosan, poly–N,N–dimethyl–N,N–diallylammonium chloride, sodium carboxymethylcellulose and aromatic copolyamide, synthesized on the bases of dichloranhydride of isophthalic acid and two diamines (4,4'–(2,2'–sodium disulfonate)–diaminodephenyl, 4,4'–(2,2'–dicarboxylic acid)–diaminodiphenylmethyl) and bovine serum albumin (BSA) in aqueous solutions were studied. It was shown that as a result of macromolecular reactions protein–polyelectrolyte complexes (PPC) forms, stabilized mainly by electrostatic forces. To characterize their composition we used the value of parameter φ, defined as an amount of ionic groups of polyelectrolytes calculated per macromolecule of protein. Using spectrophotometry and conductometry it was established that the composition of PPC in the studied systems when components are mixed at optimal conditions corresponds to the value of φ ~30 – 90. The conditions for the existence of insoluble PPC were determined. In the case of precipitation of poly-N,N–dimethyl–N,N–diallylammonium chloride and chitosan in protein – polyelectrolyte reactions the maximum yield of the product is observed at $pH \geq 7$. The introduction of sodium carboxymethylcellulose and aromatic copolyamide into the interaction with protein accompanied by shifting the range of PPC existence into the acidic region: the maximum yield of the product is observed at $pH \leq 4$. The size of the formed complex particles ranges from 10 nm ([PE]/[BSA] = 0.01 – 0.05 g/g) to ~ 1.0 – 5.5 μm ([PE]/[BSA] = 0.1 – 0.35 g/g). In case of relatively short–chained aromatic copolyamide, containing significant amount of non–ionized carboxylic groups, the ratio of micron size particles is about 5%. The presence of sulfonate and carboxylic groups in the composition of copolyamide gives an additional opportunity to regulate the conversation degree in the interpolyelectrolyte reactions, thus also implying the structure and the composition of the formed complexes.

Key words: natural and synthetic polyelectrolytes, bovine serum albumin, interpolyelectrolyte reactions

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INTRODUCTION

The reaction products received during the interaction of chemically complementary polymers are called interpolymer complexes. Hydrogen bonds, Wan–der–Waals and donor–acceptor interactions can serve as basic stabilizing forces of such compounds. Special place here belongs to the interpolyelectrolyte complexes (IPEC) stabilized by electrostatic forces. They are formed as a result of coupling of oppositely charged polymer electrolytes or during matrix polymerization of ionic monomers upon the oppositely charged polyions [1-3]. The introduction of deoxyribonucleic acid (DNA) or protein macromolecule into IPEC as a polyelectrolyte partner causes the formation of biopolyelectrolyte and protein–polyelectrolyte complexes (PPC) [4-6]. Such interactions as polyelectrolyte – biopolymer and polyelectrolyte – protein seem to be highly perspective for various applications in pharmaceutics, food industry, biomedicine including protein extraction and purification [7-10], obtaining bioactive materials, especially based on the multilayer systems [11-14], biosensors and biocatalysts development [15, 16], as well as biological substances encapsulation, medicines transfer, gene transfection and gene therapy [17-19]. The research results of the complex formation process in the protein containing systems, including both natural [20-22] and synthetic [23-25] polyelectrolytes are widely published. However, there are no deep investigations dealing with the impact character of polymer macromolecular chain rigidity and the nature of its ionic groups on such interaction as polyelectrolyte - protein, on the composition and the structure of obtained products. The research objective is to study the contribution of these factors to the complexes formation.

Bovine serum albumin was selected as a simulating protein, as it is a typical globular protein, occurring as a monomer in the solution. The interest to it as a research object is caused by its isoelectric point (pI = 4.9) allowing to change the boundary space charge of macromolecules in a reasonable pH range.

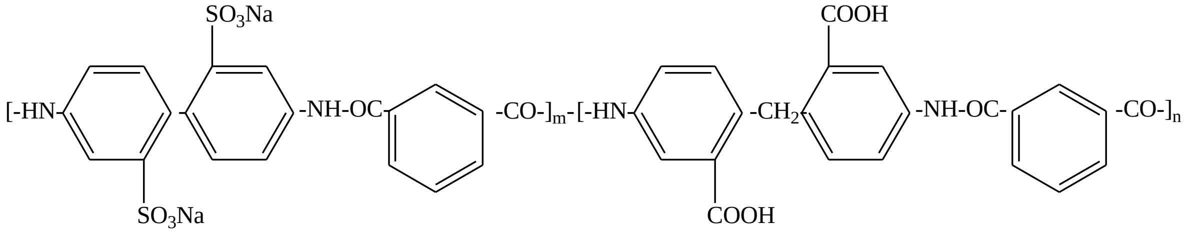
EXPERIMENTAL TECHNIQUE

Natural and synthetic polyelectrolytes: chitosan (CS) ($\overline{M_w}$ = 2.5·10^5, degree of substitution at amino groups 0.8) (ZAO Bioprogress), poly-N,N-dimethyl-N,N-diallylammonium chloride (PDMDAAC) ($\overline{M_w}$ = 1.5·10^5) (OAO Kaustik), sodium carboxymethylcellulose (CMC) mark 7M ($\overline{M_\eta}$ = 2.5·10^5, degree of substitution 0.8) (Aqualon–Hercules) and aromatic copolyamide (PA) ($\overline{M_n}$ = 3.0·10^4) (OAO Polymersintez) of the following structure:

n = 42; m = 58 mol.%

were used in the work [28].

Characteristics of bovine serum albumin (BSA) (Sigma) shown in the table.



Table

Characteristics of bovine serum albumin

***Таблица*. Характеристики бычьего сывороточного альбумина**

Protein	Molecular weight, kDa	pI	Stokes radius of the molecule, Å	Structure
Bovine serum albumin [26, 27]	67.0	4.9	34.0	Ala – 48; Phe – 30; Lys – 60; Pro – 28; Thr – 34; Cys – 35; Gly – 17; Leu – 65; Gln – 21; Val – 38; Asp – 41; His – 16; Met – 5; Arg – 26; Trp – 2; Glu – 58; Ile – 15; Asn – 14; Ser – 32; Tyr – 21

PPC solutions were obtained by mixing in a specified ratio the solutions of bovine serum albumin (0.07-0.7 g/l) and polyelectrolytes (PE) (0.001-0.01 mol/l). To prepare the protein solutions universal buffer was used.

The compositions of PPC characterized by the φ parameter defined as the number of ionic groups of polyelectrolytes per macromolecule of protein.

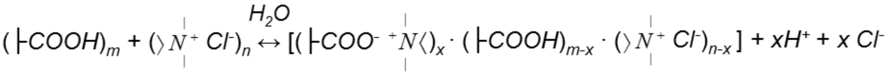
An ionomer Mettler Toledo with glass electrode as a measuring one used to carry out potentiometric titration of polyelectrolyte and PPC solutions. The change in electrical conductivity during the conductometric titration recorded using conductometer HI8733N.

A spectrophotometer SF–2000 used to measure the apparent optical density of solutions. The studies were carried out at wavelength of 440 nm. To study the phase state of PPC the 0.1 M solutions of HCl and NaOH were used.

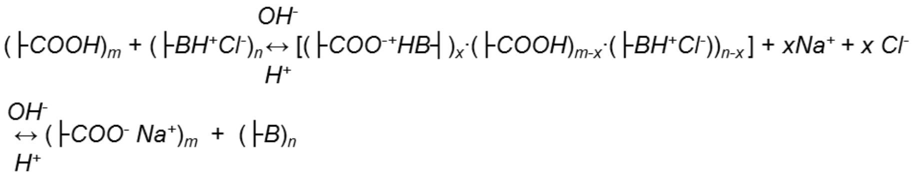
To determine the size of PPC particles laser diffractive microanalyzer HORIBA LB–550 with a source of emission as a laser diode (λ = 650 nm), photoelectric multiplier and Fourier transforms was used.

RESULTS AND DISCUSSIONS

Being polyampholytes, the proteins can react with polyelectrolytes, containing both basic (Scheme 1, 2) and acid (Scheme 3, 4) functional groups:

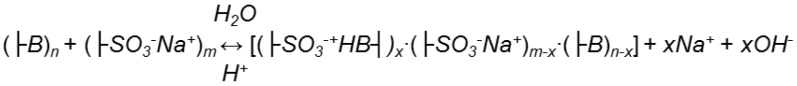


$$(\vdash COOH)_m + (\rangle \overset{|}{\underset{|}{N}}{}^{+}\, Cl^-)_n \overset{H_2O}{\leftrightarrow} [(\vdash COO^-\; {}^{+}\overset{|}{\underset{|}{N}}\langle)_x \cdot (\vdash COOH)_{m-x} \cdot (\rangle \overset{|}{\underset{|}{N}}{}^{+}\, Cl^-)_{n-x}] + xH^+ + x\,Cl^- \qquad (1)$$

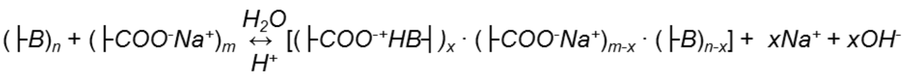


$$(\vdash COOH)_m + (\vdash BH^+Cl^-)_n \underset{H^+}{\overset{OH^-}{\leftrightarrow}} [(\vdash COO^{-+}HB\dashv)_x \cdot (\vdash COOH)_{m-x} \cdot (\vdash BH^+Cl^-))_{n-x}] + xNa^+ + x\,Cl^-$$

$$\underset{H^+}{\overset{OH^-}{\leftrightarrow}} (\vdash COO^-\, Na^+)_m + (\vdash B)_n \qquad (2)$$



$$(\vdash B)_n + (\vdash SO_3^-Na^+)_m \underset{H^+}{\overset{H_2O}{\leftrightarrow}} [(\vdash SO_3^{-+}HB\dashv)_x \cdot (\vdash SO_3^-Na^+)_{m-x} \cdot (\vdash B)_{n-x}] + xNa^+ + xOH^- \qquad (3)$$



$$(\vdash B)_n + (\vdash COO^-Na^+)_m \underset{H^+}{\overset{H_2O}{\leftrightarrow}} [(\vdash COO^{-+}HB\dashv)_x \cdot (\vdash COO^-Na^+)_{m-x} \cdot (\vdash B)_{n-x}] + xNa^+ + xOH^- \qquad (4)$$

where B: -NH; ›NH; ›N

Schemes 1, 3, 4 are implemented when strong polyelectrolytes, such as PDMDAAC, PA and CMC participate in the interpolyelectrolyte reactions (IPR), but Scheme 2, when weak polybases, such as chitosan ($pK_a = 6.3$ [2]), do.

The ability of protein to form soluble and insoluble complexes with polyelectrolytes is known to be determined by the polyelectrolytes characteristics as well as by the IPR conditions [2, 4-6]. Fig. 1 shows the regions of existence of insoluble PPC in the researched systems. When participating in the reaction of protein – polyelectrolyte PDMDAAC and CS, maximal product yield is detected at pH ≥ 7, i.e. when ζ potential of BSA macromolecules is below -18 mV [24].

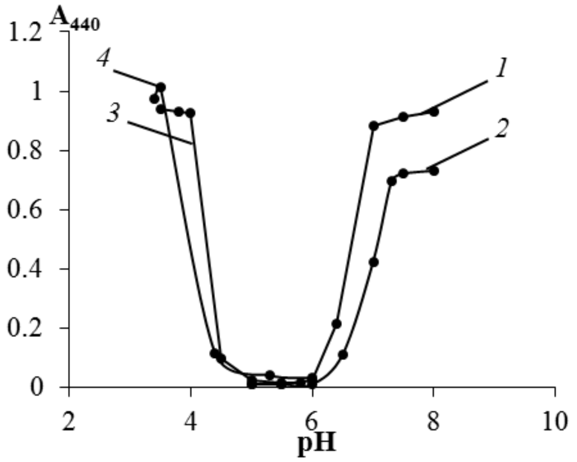


Fig. 1. Influence of pH on the phase state of complexes BSA with PDMDAAC (1), CS (2), CMC (3), PA (4); $c_{BSA} = 0.7$ g/l
Рис. 1. Влияние pH на фазовое состояние комплекса БСА с ПДМДААХ (1), ХТ (2), КМЦ (3), ПА (4); $c_{БСА} = 0,7$ г/дм^3

The introduction of CMC and PA into the interaction with protein is accompanied by shifting PPC existence range into the acidic area: the maximum product yield is observed at pH ≤ 4 (ζ potential of BSA macromolecules exceeds +12 mV [24]). It should be considered, that PA macromolecules, similar to sulphonate ones, contain carboxylic groups ($pK_a = 4.2$), capable of forming salt bonds with BSA molecules at pH range from 4.2 to 4.9.

The impact character of polyelectrolytes structure and the nature of their ionic groups on the formed PPC composition were shown in Fig. 2. The PPC composition corresponding to maximum optical density on the turbidimetric titration curves is determined by the values of φ ~ 30 for PA, ~ 60 for CMC, ~ 80 for PDMDAAC and ~ 90 for CS. These results generally correlate with the conductometric titration data, despite the fact that the amount of the forming salt bonds during the reaction between protein and polyelectrolyte, determined by the conductometric method cannot be equitable to φ value.

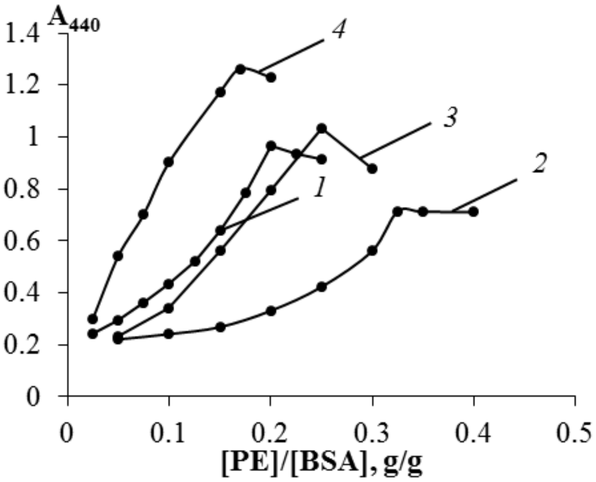


Fig. 2. Influence of PE nature on the composition of complexes BSA with PDMDAAC (1) (pH = 7.5), CS (2) (pH = 7.5), CMC (3) (pH = 3.5), PA (4) (pH = 3.5); $c_{BSA} = 0.7$ g/l
Рис. 2. Влияние природы ПЭ на состав комплекса БСА – ПДМДААХ (1) (pH = 7,5), ХТ (2) (pH = 7,5), КМЦ (3) (pH = 3,5), ПА (4) (pH = 3,5); $c_{БСА} = 0,7$ г/дм^3

It is worth mentioning, that at pH = 3.5 ionization of carboxylic groups in PA is suppressed and they are not involved in the formation of salt bonds with protein molecules. Studying the composition of insoluble complexes, formed between linear poly–N–ethyl–4–vinylpyridinium bromide and BSA, the φ ~ 55 value was received [29]. The authors indicate, that the complex composition practically equals the value of $\varphi^* = |n_+ - n_-|$, which is an absolute value of the difference between positively and negatively charged groups in the protein molecules at a given pH, which, in its turn, is determined by its amino acids composition [29]. The studied systems include rigid–chain polyelectrolytes: the value of α parameter in Kuhn–Mark–Houwink equation equals 0.81 [2], 0.85 [2] and 0.92 for CMC, chitosan, and PA respectively. However, there is no definite correspondence between the formed PPC composition and the macromolecular chain rigidity included in the polyelectrolyte complex. It can be assumed, that the conformational rearrangement of protein macromolecules at pH < 4.3 significantly influences PPC composition [30]. Besides the role of the ionization degree of polyelectrolyte functional groups should not be neglected. In case of relying on the process, suggested in the research [31], the contribution of the conformation state of polyelectrolyte macromolecules at the stage of interaction with the protein is determined mainly by the availability of polymer ionic groups (Fig. 3).

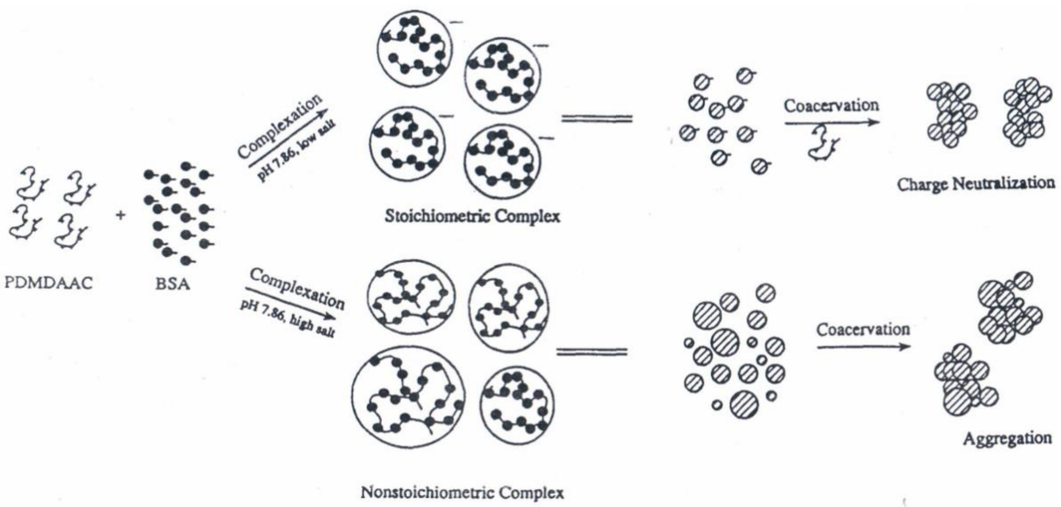


Fig. 3. Schematic diagram of complex BSA – PDMDAAC formation [31]
Рис. 3. Принципиальная схема формирования комплекса БСА-ПДМДААХ [31]

The impact character of this factor on the structure–forming processes is shown in Fig. 4.

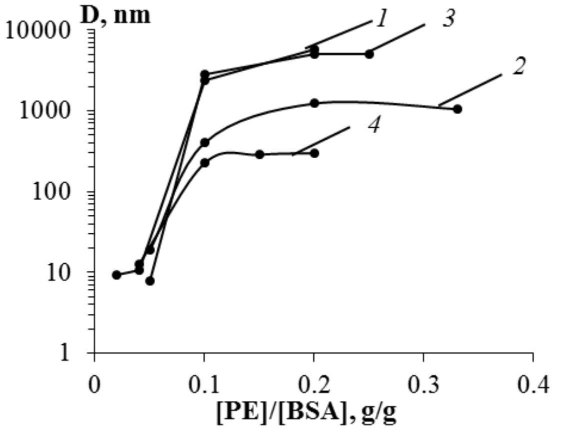


Fig. 4. Influence of the composition on the average size of the complex particles BSA with PDMDAAC (1) (pH = 7.5), CS (2) (pH = 7.5), CMC (3) (pH = 3.5), PA (4) (pH = 3.5); c_{BSA}= 0.07 g/l
Рис. 4. Влияние состава на средний размер частиц комплексов БСА – ПДМДААХ (1) (pH = 7,5), ХТ (2) (pH = 7,5), КМЦ (3) (pH = 3,5), ПА (4) (pH = 3,5); $c_{БСА}$= 0,07 г/дм^3

According to the research data [24] hydrodynamic radius of BSA molecule at the concentration of 0.1% and at pH range from 3.0 to 11.5 is about 4 nm. In the considered systems the introduction of polyelectrolyte into the solution leads to the formation of particles, which average size ranges from ~ 10.0 nm ([PE]/[BSA] = 0.01-0.05 g/g) to ~ 1.0-5.5 μm ([PE]/[BSA] = 0.1-0.35 g/g). In case of relatively short–chained PA, containing significant amount of non–ionized carboxylic groups that do not participate in the formation of salt bonds of polyelectrolyte – protein, the ratio of micron size particles is about 5% under the considered conditions.

CONCLUSIONS

The conducted research revealed that the principle factors determining the composition and the structure of protein – polylectrolyte complexes are the ionization degree of the functional groups participating in the interpolyelectrolyte reaction, determined by their nature and by the solution pH, as well as the conformation state and molecular mass of polymer electrolyte. The composition of PPC under the optimal conditions of IPR corresponds to the value of φ ~ 30-90. The size of the formed complex particles ranges from 10 nm to 5.5 μm. The presence of both sulphonate and carboxylic groups in the copolyamide composition gives an additional opportunity to regulate the conversation degree in the interpolyelectrolyte reactions, thus also implying the structure and the composition of the formed complexes.

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