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| D:\Rinat\Rinat\доки\журнал\статьи\logo.jpg | INTERACTION BETWEEN PARTIALLY BETAINATED CHITOSAN AND ALBUMIN IN ALkALESCENT MEDIA | | |
| Cite this: *INEOS OPEN*,  **2025**, *8 (1–3)*, XX–XX  DOI: 10.32931/ioXXXXx  *Received XX Month 20XX,*  *Accepted 26 September 2024*  http://ineosopen.org | | E. A. Bezrodnykh,\**a* O. V. Vyshivannaya,*a*,*b* N. A. Tishchenko,*a*  V. E. Tikhonov,*a* Y. A. Antonov,*c* V. N. Orlov,*d* and I. V. Blagodatskikh*a* | |
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| Abstract  The interaction of partially *N*-betainated oligochitosans (Q-CHIs) with bovine serum albumin (BSA) in alkalescent media is described. It is shown that Q-CHIs form complexes with BSA in solutions with pH 7.4. The isothermal titration calorimetry in buffers with different ionization heats reveals a significant contribution of the electrostatic forces to binding and the additional ionization of primary amino groups of Q-CHIs due to the proton transfer from the buffer substance. The circular dichroism study shows that the secondary structure of BSA in soluble complexes remains unaltered. | | | **pH 7.4**  N-betainoyl chitosan  N+(CH3)3  NH3+  COO**–**  COO**–**  BSA |
| **Key words:** quaternized chitosan, bovine serum albumin, complexation, isothermal titration calorimetry. | | | |

**Introduction**

Chitosan, a polyaminosaccharide derived from natural chitin, is of particular interest for biomedical applications due to its biocompatibility, biodegradability, and antimicrobial properties [1, 2]. However, a loss of solubility in neutral and alkalescent media is a limiting factor for its applicability. One approach to solving this problem is the introduction of quaternary ammonium moieties into the chitosan chain. Various methodologies have been proposed for chitosan quaternization [3, 4] to improve the solubility of chitosan in alkalescent media, mimicking the main physiological fluids and providing the complexation with polyacids, DNA, and proteins [3, 5–7]. The advantages of *N*-betainated oligochitosan [4] over other quaternized chitosan derivatives are its higher biocompatibility and lower toxicity along with the high antimicrobial activity in alkalescent media [8].

The goal of this work was to study the complexation of *N*-betainated oligochitosans with bovine serum albumin (BSA), as the main plasma protein, in alkalescent media mimicking physiological fluids and to elucidate the influence of complexation on the BSA secondary structure. The results obtained can be useful for the construction of chitosan-based pharmaceutical compositions and drug delivery systems.

Results and discussion

To gain an insight into the characteristic features of the interaction between partially *N*-betainated oligochitosan and BSA, we titrated chitosan derivative solutions into BSA solutions in various buffers and studied the interaction using light scattering (LS) and isothermal titration calorimetry (ITC). Two chitosan samples with the following characteristics were used: *M*w 14000 (Q1-CHI) and 10400 (Q2-CHI), the content of betainated units 23 (Q1-CHI) and 12 (Q2-CHI) mol %, the content of ethoxycarbonylated units 27 (Q1-CHI) and 18 (Q2-CHI) mol %.

The titration of the Q-CHIs solutions into the BSA solutions in both phosphate and TRIS buffers revealed an increase in the scattering intensity and the size of aggregates as well as an increase in the contribution of aggregates to the scattering intensity (Figu. 1), which indicated the polymer–protein interaction.

Q-CHIs contain ionizable amino groups of two types: weakly basic primary amino groups and strongly basic quaternary trimethylammonium groups along with hydrophobic *N*-ethoxycarbonyl units [4]. In this regard, considering the interaction of Q-CHI with BSA in solution with pH 7.4, it was important to clarify the main mechanism of interaction. Namely, whether amino groups of both types participate in the electrostatic binding or only strongly basic trimethylammonium groups are involved. A comparison of the ITC profiles (Fig. 2) of Q-CHI in phosphate buffer with a low heat of ionization (Δ*H* = 0.864 kcal/mol) and in TRIS buffer with a high heat of ionization (Δ*H* = 11.4 kcal/mol) evidenced the involvement of the proton transfer from the buffer substance to the chitosan derivative upon polymer–protein binding [9]. This suggests that the primary amino groups of Q-CHI also participate in the electrostatic binding of Q-CHI with BSA. Furthermore, a sharp decline in the exothermic effect of binding in phosphate buffer at a relatively high ionic strength (in the presence of 0.15 M NaCl) indicated the suppression of the additional ionization of amino groups that leads to a predominantly hydrophobic type of the polymer–protein interaction (Fig. 2).



**Figure 1.** Dependence of the relative scattered light intensity on the Q-CHI/BSA charge ratio *z* =([NH2]+[N(CH3)3])/[COOH]) in phosphate buffer at pH = 7.4 (***a***) and the particle size distributions in the mixtures with different charge ratios during the titration of Q2-CHI into BSA (***b***).



**Figure 2.** ITC profiles upon the titration of betainated chitosans into BSA at pH = 7.4: (***a***) Q1-CHI in phosphate buffer (***1***), salt phosphate buffer (***2***), and TRIS/HCl buffer (***3***), (***b***) Q2-CHI in phosphate buffer.

To elucidate the effect of Q-CHI binding to BSA on the protein secondary structure, the soluble complexes were studied by circular dichroism (CD). The results obtained (Table 1) suggest the negligible alteration of the BSA structure in the presence of the chitosan derivative.

Table 1. Secondary structures of BSA and BSA bound to Q2-CHI

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| Sample | α-helix | β-sheet | β-turn | Unordered |
| BSA | 63 | 6 | 10 | 21 |
| Q2-CHI/BSA  *z* = 0.04 | 61 | 7 | 11 | 21 |
| *z* = 0.06 | 61 | 7 | 11 | 21 |
| *z* = 0.12 | 63 | 7 | 10 | 20 |

Experimental section

The *N*-betainated oligochitosan samples were prepared using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline as a coupling agent according to the published procedure [4]. The composition was determined by 1H NMR spectroscopy [4], the molecular weight characterization was performed using GPC [10].

The LS studies were carried out on a Photocor Complex spectrometer (Russia) [10].

The ITC analysis was performed with a MicroCal VP-ITC titration microcalorimeter (USA) at 25 °C [10]. For both the LS and ITC experiments, the solutions of Q-CHIs with a concentration of [NH2] + [N(CH3)3] = 3 mM and BSA solutions with a concentration of [COOH] = 0.6 mM in phosphate and TRIS buffers with pH 7.4 and an ionic strength of 20 mM were used.

The CD spectra of the BSA solution and Q-OCHI/BSA mixtures in phosphate buffer (pH = 7.4) with an ionic strength of 20 mM were recorded at 21 °C using a Chiroscan Applied Photophysics instrument. The protein concentration was 0.01%.

**Conclusions**

The LS and ITC studies showed that partially *N*-betainated oligochitosan forms complexes with BSA in solutions with pH 7.4 and their binding has the electrostatic nature. During the complexation, the interaction of both quaternary and primary amino groups occurs due to the proton transfer from the buffer substance to the chitosan derivatives. The CD studies revealed that the secondary structure of BSA in soluble complexes remains unaltered. An increase in the ionic strength to 0.15 M suppresses the electrostatic binding.

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