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Structure of bovine serum albumin in solution and films as revealed from vibrational spectroscopy

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Abstract. This study explores the impact of salt concentration and the degree of hydration on the structure of bovine serum albumin (BSA) using vibrational spectroscopy methods, specifically Fourier transform infrared spectroscopy and Raman scattering. BSA, a key plasma protein, plays essential roles in binding and transporting various molecules in the bloodstream. The research focuses on understanding how do the interaction with ions and water molecules affect the secondary and tertiary structure of globular proteins, emphasizing the significance of environmental factors in protein conformation. The results indicate distinct responses in the vibrational spectra of BSA to the presence of salt. Analysing the Amide I band give the parameters of the secondary structure of BSA. In all systems investigated the values obtained is in good correspondence with the data for native BSA, but the secondary and tertiary BSA structure in dehydrated films containing NaCl is closer to native, hence ions prevent albumin from denaturation and β-aggregation.

Keywords: bovine serum albumin, protein film, Raman spectroscopy, FTIR spectroscopy

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Структура бычьего сывороточного альбумина в растворе и пленках по данным колебательной спектроскопии

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Аннотация. В данной работе изучается влияние концентрации соли и степени гидратации на структуру бычьего сывороточного альбумина (БСА) с помощью методов колебательной спектроскопии, в частности, инфракрасной спектроскопии с преобразованием Фурье и комбинационного рассеяния. БСА, ключевой белок плазмы крови, играет важную роль в связывании и транспортировке различных молекул в кровотоке. Исследование посвящено изучению того, как взаимодействие с ионами металлов и молекулами воды влияет на вторичную и третичную структуру глобулярных белков, подчеркивая значимость факторов окружающей среды для конформации белка. Полученные результаты свидетельствуют о том, что в колебательных спектрах БСА наблюдается отчетливый отклик на присутствие соли. Анализ полосы Амид I позволяет получить параметры вторичной структуры БСА. Во всех исследованных системах полученные значения хорошо согласуются с данными для нативного БСА, но вторичная и третичная структура БСА в высушенных пленках, содержащих NaCl, ближе к нативной, следовательно, ионы предотвращают денатурацию и β-агрегацию альбумина.

Ключевые слова: бычий сывороточный альбумин, пленки белка, рамановская спектроскопия, ИК-Фурье-спектроскопия

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Introduction

A wide variety of approaches based on vibrational spectroscopy have been used to study protein structure. The advantages of this method are a small amount of sample, the ability to study multicomponent systems and complex structures (such as cells, tissues, biological fluids), and the use of a substance in the liquid or solid phase. FTIR and Raman scattering spectroscopy provide information about the secondary and tertiary structure of proteins, their conformational transitions in consequence of folding, variation in external conditions or intermolecular interactions [1–3]. Methods for diagnosing various diseases are now being actively developed, based on measuring the vibrational spectra of blood, hair and other biological samples, followed by analysis of the structure of biomolecules as well as Principal Component-Discriminant Function Analysis [1, 4–8]. Spectroscopy investigation of biofluids is carried out in transmission mode or with ATR technique and often includes preliminary dilution or evaporation of samples [7, 8]. Since commonly the assay is mainly based on spectral features of proteins, it is important to take into account changes in structure and spectral parameters of proteins upon variation of water activity. The present work is devoted to comparison of the IR and Raman spectra of bovine serum albumin (BSA) in solutions and dried films to analyze influence of water and monovalent ions content on conformation parameters of the protein.

BSA, a prevalent plasma protein, is extensively utilized for studying various aspects of protein behavior, such as folding and aggregation, as well as for biotechnological purposes [9]. BSA primarily functions in binding, transporting, and delivering a wide array of small molecules and metal ions in the bloodstream. Structurally, BSA consists of a single polypeptide chain organized into three domains (I, II, III), with a dominant α-helical secondary structure at room temperature [10].

Materials and Methods

BSA lyophilized powder (DiaM, USA) was dissolved in deionized water or 0.15M NaCl solution in concentration of 70 g/l. IR spectra of albumin solutions and films were recorded on FTIR spectrometer Nicolet 8700 (Thermo Scientific), on ATR attachment. 10 μl of BSA solution was dropped on the ATR crystal and covered with a cap to prevent evaporation during the recording. The spectrum of the background was recorded and subtracted from the spectra of the samples automatically. The spectrum of the corresponding solvent was registered for further subtraction from the spectra of protein solutions. BSA dehydrated films were obtained from protein solutions dried by a nitrogen stream. The spectral data within the range of 4000 to 500 cm^{-1} were recorded, and 512 scans were averaged for each spectrum with a spectral resolution of 2 cm^{-1}. The software supplied with the spectrometer and OriginPro were used for data processing.

Raman spectra of BSA dehydrated films prepared on aluminium foil were measured by Express Raman spectrometer SENTERRA (Bruker). The excitation source was 785 nm laser, the spectral resolution was 3 cm^{-1}. The laser power on the sample was about 100 mW. The curve fitting analysis was implemented using the OPUS/IR v 5.0 program and Origin Pro.

Results and Discussion

The BSA IR spectra were measured in water solution and in 0.15 M NaCl, as well as in films obtained by dehydration of these solutions. Raman spectra of BSA were recorded in films prepared from the same solutions by desiccation of 10 μl drop on aluminium foil. In the IR and

Raman spectra of proteins, the vibrational band of the Amide I peptide group (about 1650 cm^{-1}) is very sensitive to various forms of secondary structure [11]. After the baseline correction, we analyse the Amide I band by procedure of decomposition on components of Gaussian contours. The area of each Gaussian contour represents the contribution of the definite type of the secondary structure [11]. The percentage of the components of BSA secondary structure in the studied systems is given in Table 1. According to literature data, the secondary structure of BSA is composed of 67% α-helix, 10% turn, and no β-sheet is contained [3, 10, 12]. From the FTIR spectroscopy results we can conclude that after desiccation the content of α-helices in protein secondary structure grows and the content of β-sheets decreases. BSA structure in dehydrated films containing NaCl is closer to native, so we can conclude that ions Na^+ and Cl^- prevent albumin from denaturation and β-aggregation.

Table 1

Content of BSA secondary structure forms in solutions and films

	Film and solution composition	α-helices, %, ±4% (band position, cm^{-1})	β-sheets, %, ±4% (band position, cm^{-1})	β-turns, %, ±4% (band position, cm^{-1})
Raman spectra	water (film)	65 (1655)	1 (1636)	25 (1671, 1683)
	NaCl (film)	66 (1655)	4 (1693)	22 (1670, 1682)
Infrared spectra	water (solution)	58 (1653)	36 (1628)	6 (1680)
	NaCl (solution)	54 (1652)	23 (1630)	8 (1680)
	water (film)	63 (1648)	14 (1632)	7 (1681)
	NaCl (film)	70 (1650)	5 (1632)	15 (1677)

Raman spectrum of a protein along with the vibrational bands of peptide group contains strong signals from amino acids side chains, and it was found to be very sensitive to their conformations and surroundings. Raman spectra of BSA films after baseline correction and normalization on the Amid I intensity with assignments of the bands are shown in Fig. 1. Several vibrational modes can be used to analyze BSA structure (Table 2) [1, 2]. Change in the intensity ratio of 850 cm^{-1} to 827 cm^{-1} talks about alteration in manner of H-bonding of phenyl hydroxyl of the tyrosine.

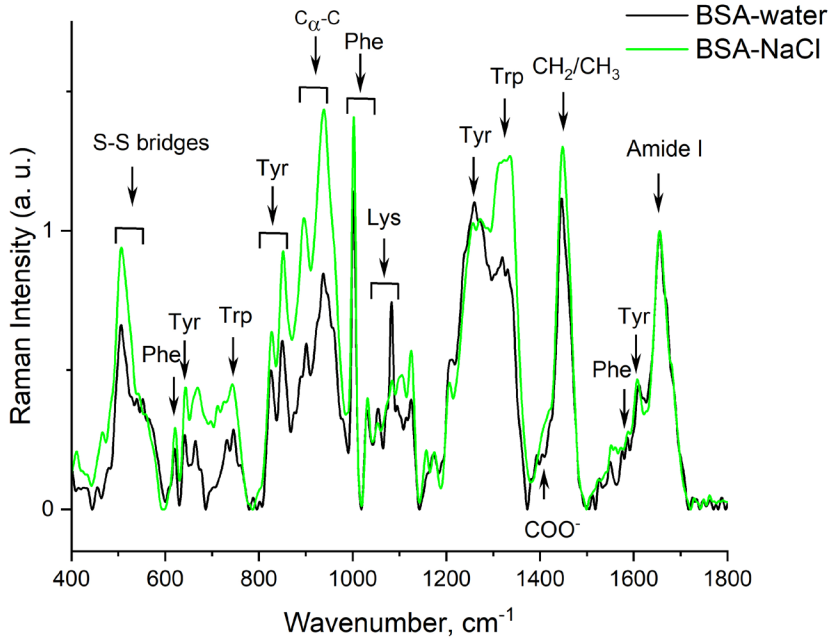

Fig. 1. Raman spectra of BSA films obtained from water and from 0.15M NaCl solution

The intensity of Phe band 621 cm^{-1} lowers at the degree of hydration increases. The ratio of Trp doublet I_{1360}/I_{1340} decreases at the transition of Trp into more polar medium. The value I_{1177}/I_{1003} reduces if the water content around the Tyr residue curtails. All these markers show the lowering of degree of hydration of non-polar amino acids in NaCl-containing film in comparison with film, prepared from water solution. This may indicate a more preserved tertiary structure of albumin in the NaCl-containing film.

Table 2

Protein structure markers from Raman spectra of BSA

Structure markers		$\frac{I_{621}}{I_{1003}}$, **Phe**	$\frac{I_{1360}}{I_{1340}}$, **Trp**	$\frac{I_{850}}{I_{827}}$, **Tyr**	$\frac{I_{1177}}{I_{1003}}$, **Tyr**
Film composition	water	0.187	0.305	1.21	0.172
	NaCl	0.208	0.337	1.45	0.146

Conclusion

In summary, the experimental study involving the measurement of protein spectra in different conditions highlights the intricate sensitivity of vibrational bands to variations in salt concentration and humidity level. The investigation of aromatic amino acids vibrations reveals the influence of salt on the protein environment, indicating a shift towards a less polar state. BSA structure in dehydrated films containing NaCl is closer to native. These findings shed light on the structural alterations of proteins in response to varying conditions and provide insights into the interplay between environmental factors and protein conformation.

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