Application of FTIR and Raman Spectroscopy to Study the Structure of Serum Albumin in Solutions and Films

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Abstract: - Various vibrational spectroscopy methods have great potential for use in disease diagnostics. The development and standardization of such techniques is an urgent biomedical task. This work is devoted to comparing the capabilities of ATR FTIR spectroscopy and Raman scattering for studying the structure of proteins in solutions and films of various compositions. In this work, the parameters of the secondary structure of bovine serum albumin (BSA) are determined in water and 0.15M NaCl solutions as well as in films made by the drying of these solutions. It is shown that α-helix quantity in BSA increases, and the amount of β-sheets is reduced after the dehydration. Analysis of Raman peaks from aromatic amino acids indicated a lowering of their degree of hydration in NaCl-containing film in comparison with film, prepared from water solution. This allows us to conclude that the protein structure is closer to the native globule in the presence of NaCl. Despite the fact that the BSA structure can be considered native in all the conditions studied, we should note that different water and salt contents in the sample affect the spectral properties and conformational state of the protein.

Key-Words: - Vibrational spectroscopy, protein secondary structure, Amide I band, serum albumin, hydration, native globule, partial denaturation

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1 Introduction

At the present time methods for diagnosing various diseases aimed at broad population coverage are being actively developed, [1], [2], [3], [4]. Many new approaches are based on measuring the vibrational spectra of different biological samples: blood (blood serum), nails, skin, etc., with subsequent analysis of the structure of biomolecules, [4], [5], [6], [7], [8], [9]. IR Fourier spectroscopy and Raman scattering have a number of advantages that are important for use in mass screening: non-invasiveness, rapid registration and high information content of the spectrum, as well as the use of both liquid and solid samples, the ability to study multicomponent systems and complex structures. Spectroscopic studies of biofluids are carried out using scattering, transmission or ATR methods and often include preliminary dilution or evaporation of samples, [6], [10]. Since the analysis is usually based mainly on the spectral features of proteins, it is important to take into account changes in the structure and spectral parameters of proteins with a change in water activity. The present work is devoted to the comparison of IR and Raman spectra of bovine serum albumin (BSA) in solutions and dried films in order to analyze the effect of water and NaCl on the conformational parameters of the protein.

Serum albumin is the most prevalent protein in the circulatory system (70% of the total protein composition). It performs many physiological functions, such as maintaining the osmotic pressure and pH of the blood, and also serves as a carrier, transporting a large number of endogenous and exogenous compounds, such as fatty acids, bile acids, bilirubin, lipid hormones, and some drugs, [11]. Serum albumin is widely used as a model globular protein. It is highly soluble owing to high negative charge asymmetrically distributed to its surface at neutral pH. A decrease in the charge density on its surface when varying the pH or adding a low-molecular electrolyte leads to increased protein aggregation, [12], [13], [14]. BSA consists of a single nonglycosylated chain of 607 amino acids, among them 35 cysteines which form 17 disulfide bridges, its molecular mass is 66.4 kDa.

The secondary structure of serum albumin is predominantly α-helical. 67% α-helix, no β-sheets, 10% β-turns and 23% extended chains were found by the X-ray crystallography for HSA, and close

values for BSA, [11]. In [15] a 65% α-helix content for serum albumin based on sequence comparisons of several albumins and further estimated a β-sheet and β-turn percentages of 10% and 19%, respectively were predicted. Although there are no β-sheets in the structure of serum albumin, ~23% of the structure is in an extended chain conformation, which would be predicted as β-strand, and ~10% of the remaining structure exists in turns, [16]. At the same time the secondary structure data revealed from circular dichroism and IR spectroscopy studies sometimes differ from the above-mentioned values: the content of α-helix ranges from 48% to 65%, and estimations for β-sheets can reach 40%, [17], [18], [19], [20]. It is obvious that sample preparation, protein concentration and the presence of admixtures influence the determined spectral parameters, and these factors should be carefully controlled during the measurement process to obtain reliable and reproducible results. The aim of this work is to compare the structural parameters of BSA (in this case serum albumin will be used as a model globular protein) found from the analysis of the vibrational spectra of the protein in water and water-salt solutions, as well as in films obtained from these solutions. We also compare the data revealed from FTIR and Raman scattering spectra. Both of these methods allow us to determine the secondary structure of the protein by deconvolution of the Amide I band, which is determined by the vibrations of the peptide group, but the Raman spectrum of the protein also contains strong signals from the side chains of amino acids, which are very sensitive to their conformation and environment, [21]. Another advantage is that water makes a weak contribution to the Raman spectrum, which helps to avoid errors due to incorrect subtraction of the solvent spectrum from the spectrum of the biological sample, [22].

2 Experimental

2.1 Materials

Solutions were prepared by dissolving BSA in lyophilized form (Dia-M) in bidistilled water. The protein concentration in the solution was determined spectrophotometrically taking the molar extinction coefficient of BSA ε(280nm)=43·103 $M^{-1}cm^{-1}$, [23]. Reagent-grade NaCl was used for saturated solution with subsequent dilution. Partial denaturation of BSA was carried out by maintaining the protein solution at a temperature of 60ºC for 5 min. Dehydrated BSA film were prepared by drying the 20 μl drop of protein solution with a N_2 stream.

2.2 FTIR Spectra Measurement

The spectra were obtained using a FTIR spectrometer Nicolet 8700 (Thermo Scientific) by the ATR method, [22]. 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 protein concentration in solutions was $6 \cdot 10^{-4}$ M. During the measurement the solutions were covered with a cap to prevent evaporation. BSA films were prepared from the corresponding solutions by drying the 20 μl drop of protein solution with a N_2 stream on the ATR crystal. 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 samples. The software supplied with the spectrometer and OriginPro were used for data processing.

2.3 Raman Scattering

Raman spectra were measured on a SENTERRA express Raman spectrometer (Bruker). The excitation source was a red laser (785 nm), the spectral resolution was 3 cm^{-1}. The laser power was about 100 mW. Aluminum foil was used as a substrate for BSA films. The software supplied with the spectrometer and OriginPro were used for data processing.

3 Results and Discussion

FTIR spectroscopy is well established experimental technique for the analysis of secondary structure of proteins. The most sensitive spectral region to the protein secondary structural components is the Amide I band (1700-1600 cm^{-1}), which is due almost entirely to the C=O stretch vibrations of the peptide linkages (approximately 80%). The frequencies of the Amide I band components are found to be correlated closely to the each secondary structural element of the proteins, [22], [24]. The obtained averaged IR absorption spectra were smoothed by FFT filtering and then the area between 1700 and 1600 cm^{-1} was isolated for further analysis. After the baseline correction, the decomposition of the Amid I band to components of Gaussian contours was performed. The points of minimum of the second derivative determine centers of the Gaussians. The area of each Gaussian contour represents the contribution of the definite type of the secondary structure, [24], [25].

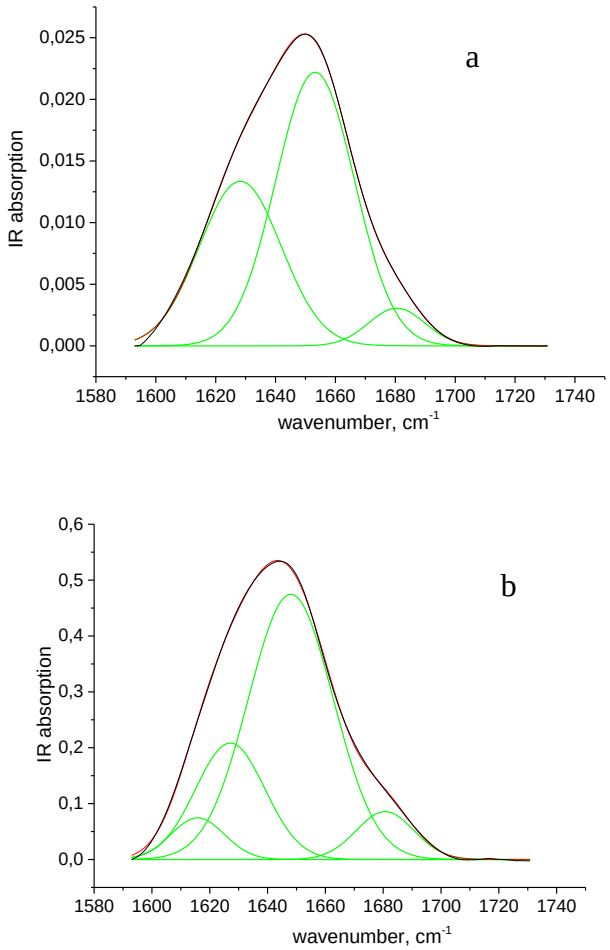

Fig. 1: Decomposition of Amid I band in IR spectra of BSA in water solution (a) and film prepared from this solution (b)

The examples of the decomposition of Amid I band in IR spectra of BSA in water solution and corresponding film are presented in Figure 1. Let us consider the shape of the Amide I band in solutions and films with different electrolyte contents (Figure 2). One can see that an increase in the salt concentration causes a shift of Amide I towards lower wavenumbers in solutions, and vice versa in the corresponding films. In solutions of partially denatured protein, an even more distinct shift of the Amide 1 band towards lower wavenumbers is observed; in the corresponding films, a shoulder appears in this spectral region of Amide 1.

The results of deconvolution of the Amide 1 band in the studied systems are given in Table 1. It can be seen that in the solution, with an increase in the NaCl concentration, the percentage of α-helices decreases, however, this does not happen in the films. In the presence of NaCl in solutions and films, the protein structure is more severely disrupted upon heating.

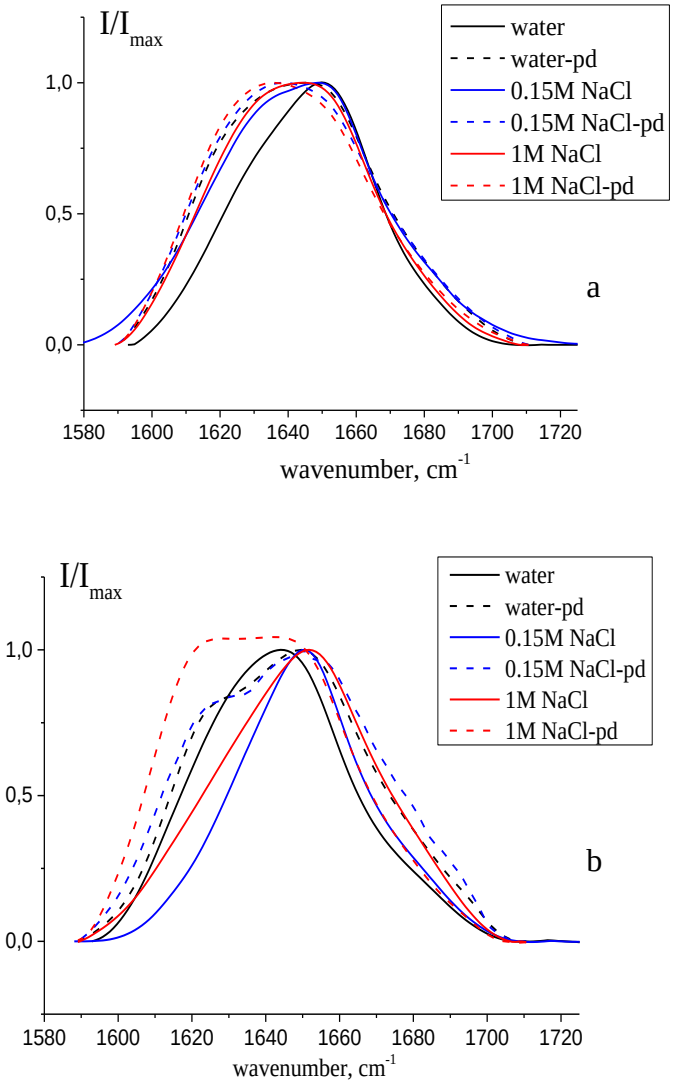

Fig. 2: Amid I bands in IR spectra of native and partially denatured (pd) BSA in solutions (a) with different NaCl concentrations and films (b) prepared from these solutions. The intensity of each band was normed on its maximal intensity.

Raman spectra of BSA in dehydrated films prepared from water and 0.15M NaCl solution after a baseline correction are presented in Figure 3. In these spectra the Amide I bands are also well distinguished; their decomposition to components of Gaussian contours was carried out analogously (Figure 4). Parameters of BSA secondary structure obtained from Raman spectra of BSA in studied systems are given in Table 2. One can see that the content of the main forms of the protein secondary structure in a water medium is in good agreement with the literature data for native serum albumin [11], [16], [17], [18], [19], [20], but it is worth mentioning some features.

Table 1. Parameters of the BSA secondary structure obtained from FTIR spectra of solutions and films. The bands assignment is according to [24], [25]

Solvent (condition of the sample)	α-helices, %, ±4% (Band position, cm^{-1})	β-sheets, %, ±4% (Band position, cm^{-1})	β-turns, %, ±4% (Band position, cm^{-1})
Native			
water (solution)	**58** (1653)	**36** (1628)	**6** (1680)
0.15M NaCl (solution)	**54** (1652)	**23** (1630)	**8** (1680)
1M NaCl (solution)	**48** (1651)	**37** (1627)	**9** (1678)
water (film)	**63** (1648)	**14** (1632)	**7** (1681)
0.15M NaCl (film)	**70** (1650)	**10** (1621) **5** (1632)	**15** (1677)
1M NaCl (film)	**67** (1653)	**16** (1628)	**10** (1681)
Partially denatured			
water (solution)	**51** (1650)	**22** (1629)	**12** (1678)
0.15M NaCl (solution)	**41** (1651)	**32** (1629)	**13** (1676)
1M NaCl (solution)	**36** (1649)	**44** (1626)	**16** (1671)
water (film)	**58** (1651)	**30** (1623)	**12** (1682)
0.15M NaCl (film)	**28** (1662)*	**27** (1622) **30** (1644) **3** (1693)	**9** (1680)
1M NaCl (film)	**41** (1649)	**41** (1623) **3** (1687)	**11** (1670)

*$(1663\pm3)cm^{-1}$ *is assigned to* 3_{10} *Helix [24]*

FTIR data show that the helicity of native BSA increases, and the amount of β-sheets is reduced as a result of dehydration in water and water-salt environments. The position of the Gaussian's center corresponding to the α-helix in IR spectra of BSA films has minimal values from the interval determined for this type of structure, whereas in the Raman spectra, the center of this Gaussian is located near the middle of the permissible interval, [25]. In general, the Raman spectrum shows better band resolution in the Amide I region than the IR spectrum (Figure 1 Figure 2, Figure 3, Figure 4). However, it is worth taking into account that in the Raman spectrum, the Amide I band is superimposed by fairly intense bands of side chains (in particular, Tyr), which makes it difficult to decipher the forms of the secondary structure.

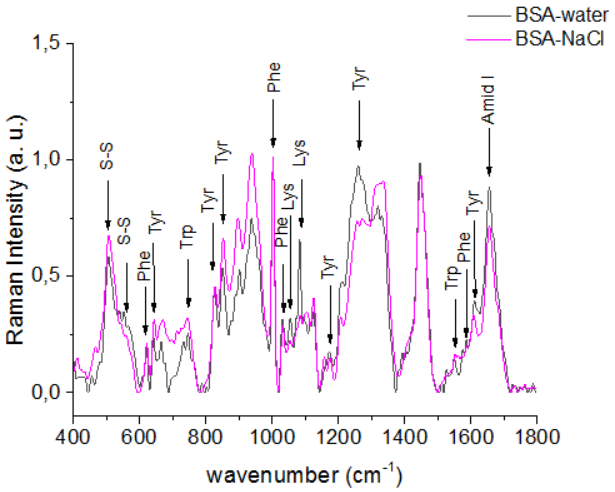

Fig. 3: Raman spectra of BSA in dehydrated films prepared from water and 0.15M NaCl solution. The bands assignment is according to [21]

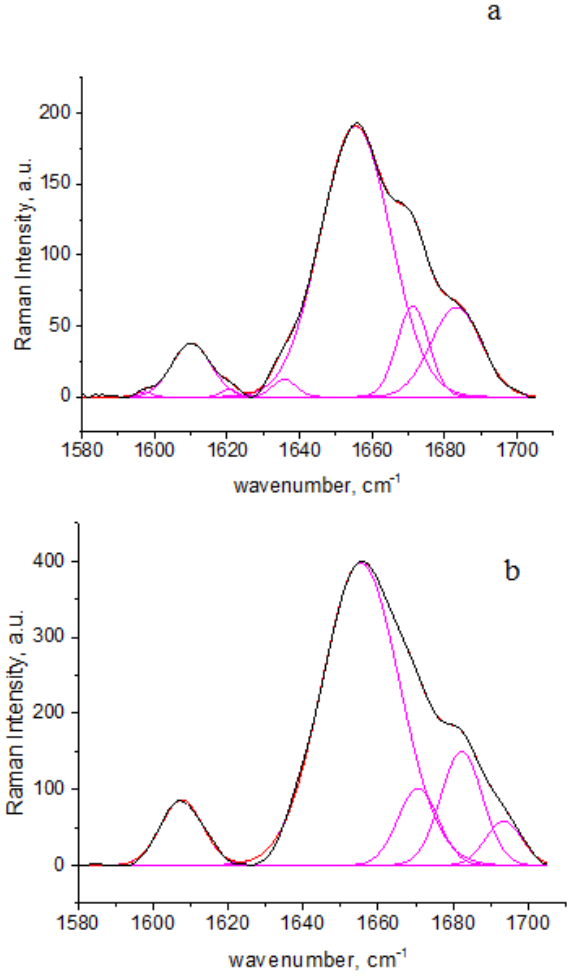

Fig. 4: Decomposition of Amid I band in Raman spectra of BSA in films prepared from water solution (a) and 0.15M NaCl solution (b)

The Raman spectrum of a protein along with the vibrational bands of peptide group contains strong peaks from amino acids side chains (Fig.3), and there were found to be very sensitive to their conformations and surroundings, [21]. Considering the positions of these peaks we can observe the influence of NaCl and heating on the following groups of BSA: S-S bridges, Phe, and CH_2/CH_3 groups (Table 3). In addition, it should be noted that there are changes in the intensity of some Raman bands related to vibrations of aromatic amino acids (Table 4). Alteration in the intensity ratio of 850 cm^{-1} to 827 cm^{-1} talks about modification in the manner of H-bonding of phenyl hydroxyl of the tyrosine. The intensity of the Phe band 621 cm^{-1} decreases as 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, [21], [26].

Table 2. Parameters of the BSA secondary structure obtained from Raman spectra of films. The bands assignment is according to [17]

Solvent (condition of the sample)	α-helices, %, ±4% (Band position, cm^{-1})	β-sheets, %, ±4% (Band position, cm^{-1})	β-turns& random%, ±4% (Band position, cm^{-1})
Native			
water (film)	**65** (1655)	**16** (1683)	**10** (1671)
0.15M NaCl (film)	**66** (1655)	**13** (1682)	**9** (1671)
1M NaCl (film)	**35** (1651)	-	**26** (1673)
Partially denatured			
water (film)	**25** (1653)	**26** (1665) **5** (1687)	**20** (1637) **12** (1676)
0.15M NaCl (film)	**34** (1654)	**16** (1689)	**4** (1639) **29** (1670)
1M NaCl (film)	**26** (1657)	**3** (1682)	**11** (1637) **29** (1671)

Table 3. Peaks position in Raman spectra of BSA films

Band assignment		S-S bridges	Phe	CH_2/CH_3
Native				
Film com-po-sition	water	506 cm^{-1} 539 cm^{-1} 552 cm^{-1} 565 cm^{-1}	1003 cm^{-1}	1446 cm^{-1}
	0.15M NaCl	507 cm^{1} 543 cm^{-1} 558 cm^{-1}	1003 cm^{-1}	1448 cm^{-1}
	1M NaCl	514 cm^{1} 554 cm^{1} 564 cm^{1}	1008 cm^{1}	1456 cm^{1}
Partially denatured				
Film com-po-sition	water	509 cm^{-1} 539 cm^{-1} 554 cm^{-1} 566 cm^{-1}	1002 cm^{-1}	1446 cm^{-1}
	0.15M NaCl	506 cm^{1} 525 cm^{-1} 549 cm^{-1} 563 cm^{-1} 574 cm^{-1}	1002 cm^{-1}	1449 cm^{-1}
	1M NaCl	508 cm^{1} 555 cm^{1} 576 cm^{1}	1002 cm^{1}	1445 cm^{1}

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. Considering that in a water-soluble globular protein aromatic amino acids are located inside the globule and are hidden from contact with the solvent, it can be concluded that an increase in the polarity of the aromatic amino acids' environment indicates a violation of the tertiary structure of the protein. So the data obtained indicate a more preserved globular structure of albumin in the NaCl-containing film.

Table 4. Relative intensities of the peaks in Raman spectra of BSA films

Band assignment		$\frac{I_{621}}{I_{1003}}$, **Phe**	$\frac{I_{1360}}{I_{1340}}$, **Trp**	$\frac{I_{850}}{I_{827}}$, **Tyr**	$\frac{I_{1177}}{I_{1003}}$, **Tyr**
Native					
Film com-po-sition	water	0.187	0.305	1.21	0.172
	0.15M NaCl	0.208	0.337	1.45	0.146
	1M NaCl	0.150	0.378	1.34	0.122
Partially denatured					
Film com-po-sition	water	0.281	0.240	3.67	0.206
	0.15M NaCl	0.203	0.321	1.11	0.165
	1M NaCl	0.290	0.602	1.21	0.311

4 Conclusion

IR and Raman spectroscopy provide broad opportunities for studying the structure of proteins, including those in complex biological systems, and in different states of samples. The development of new techniques for early diagnostics of diseases based on vibrational spectroscopy methods requires standardization of the conditions for preparing analyzed biosamples since different water and salt contents in the sample affect the spectral properties and conformation of the protein. IR and Raman spectra make it possible to determine the secondary structure of a protein; Raman spectra also contain rich information about the environment of protein side chains.

In this work the data obtained from IR and Raman spectra of BSA in solutions and films were compared; the parameters of the secondary structure of the protein found by these two methods are in good agreement with each other and with literature data. The α-helix content of BSA increases, and the amount of β-sheets is reduced after the dehydration. Analysis of Raman peaks from aromatic amino acids side chains has shown lowering of the degree of hydration of non-polar amino acids in NaCl-containing film in comparison with film, prepared from water solution, which indicates that the protein structure is closer to the native globular structure in the presence of NaCl in physiological concentration.

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Conflict of Interest

The authors have no conflicts of interest to declare.