BOOK of ABSTRACTS

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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 salt concentration variations, with the Amide I band being particularly sensitive in different conditions. It is shown that 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.

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. 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 proteins [1]. Therefore, an important task is to study the influence of the degree of hydration and the ionic composition of the medium on the secondary and tertiary structure of globular proteins. In this work, the structure of bovine serum albumin (BSA) in solutions and films is studied by varying NaCl concentration using Fourier transform infrared spectroscopy and Raman scattering.

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. 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.

Materials and Methods

BSA (DiaM, USA), NaCl reagent grade.

BSA dehydrated films were obtained from protein solutions dried by a nitrogen stream. Raman spectra were measured by Express Raman spectrometer SENTERRA (Bruker). The excitation source was a green and red laser (532, 785 nm), the spectral resolution was 3 cm^{-1}. The laser power on the sample was about 10 and 100 mW. The curve fitting analysis was implemented using the OPUS/IR v 5.0 program and Origin Pro.

IR spectra of albumin solutions and films were recorded on IR Fourier spectrometer Nicolet 8700 (Thermo Scientific), on ATR attachment, with a resolution of 2 cm^{-1}.

Results and Discussion

The protein IR spectra were measured in aqueous solutions with varying NaCl concentrations, as well as in films obtained by drying these solutions. In the vibrational spectra of proteins, the band of peptide group Amide I (about 1650 cm-1) is very sensitive to various forms of secondary structure [2]. The presence of salt causes a hypsochromic shift of this band, and in films under low humidity conditions this shift is more pronounced. 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.

Table 1

Bands assignments for Raman spectra of BSA

		Wavenumbers, cm^{-1}					
Film composition	water	507	557, 570	896, 937	960	1408	1448
	NaCl	503	563	896, 937	-	1404	1444
Band assignment		S-S bridges	S-S bridges	Cα-C, α-helix	Cα-C β-sheet	COO^-	CH_2/CH_3

Table 2

Protein structure markers from Raman spectra of BSA

Structure markers		$\frac{I_{1650}}{I_{1615}}$, $\frac{\%\alpha}{\%\beta}$	$\frac{I_{1365}}{I_{1330}}$, Trp	$\frac{I_{850}}{I_{828}}$, Tyr
Film composition	water	2.72	0.09	0.75
	NaCl	3.48	0.14	0.44

Several vibrational modes can be used to analyze BSA structure (Tables 1,2). One can see that in water media the content of β-sheets is larger then in the presence of NaCl. Analysis of the bands corresponding to vibrations of aromatic amino acids showed that in the films in the presence of salt they are in a less polar environment. Changes observed also in positions of S-S bridges, indicating aggregation process. We can conclude that BSA structure in dehydrated films containing NaCl is closer to native, i.e. ions prevent albumin from denaturation and β-aggregation.

Conclusion

In summary, the experimental study involving the measurement of protein spectra in different conditions highlights the intricate sensitivity of vibrational bands, particularly the Amide I band, to variations in salt concentration and humidity levels. The observed hypsochromic shift in the Amide I band in the presence of salt, especially accentuated in low-humidity film conditions, underscores the impact of environmental factors on protein structure. The investigation into aromatic amino acids vibrations further 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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REFERENCES

1. **Chernyshev D.A., Mikhailets E.S., Telnaya E.A.**, et. al, Analysis of infrared spectra of blood serum of patients with multiple myeloma, Journal of Physics: Conference Series, 2103 (2021) 012052, doi:10.1088/1742-6596/2103/1/012052.

2. **Barth. A**. Infrared spectroscopy of proteins. Biochimica et Biophysica Acta, 1767 (2007) p. 1073–1101.