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The hydration of Concanavalin A studied by infrared spectroscopy

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The influence of a stepwise hydration on the secondary structure of Concanavalin A, a 273 amino acid residues long lectin protein, was monitored by infrared spectroscopy. An analysis of Amide I and Amide III bands, assignment of model bands and determination of the populations of secondary structure elements using computed integral intensity of particular amino bands, was used to determine the proportion of β-sheet in protein films recorded under various steps of hydration and solution in water. In dry protein film 53% of amino acid residues are in β-sheet conformation. The hydration increases a population of β-sheet to 57% determined in fully hydrated film which finally reached 61% in water solution. On the basis of characteristic differential spectra calculated from the various hydration levels, we established that in the initial stage of hydration water molecules bind through hydrogen bonds directly to the main and side chains of protein. Hydration of side chains mainly occupies COO^- and COOH groups. The increase of β-sheet population induced by hydration rearranged the water molecules attached to COOH side chain groups. A parallel analysis of Amide III bands shows that the Amid III region provides more complete information regarding the protein structure. Contemporary analysis of this region is very supportive, because it offers additional structural parameters which significantly contribute to reliability of secondary structure analysis by applying Amide I mode. Moreover, besides the comparable information about the population of secondary structure elements, the analysis of the Amide III area provides also the distribution of conformations of amino acids which are found in unstructured parts of protein.
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1. Introduction

Protein hydration is intimately connected to the protein activity and its three-dimensional structure. It is therefore an important mechanism, which governs the structure and the dynamics of the protein. Water molecules, due to their smallness, polarity, conformational flexibility, and ability to perform as proton donor and acceptor, form an integral part of most protein structures and interactions with molecules such as ligands, DNA, or other proteins. Water molecules bind to particular protein groups by forming hydrogen bonds (H-bonds). H-bonds have a huge role in the structure of biomolecules and there are not many techniques suitable for their detection. A method that is particularly sensitive to formation of H-bonds is infrared (IR) spectroscopy [1–4]. Thus, IR spectroscopy has become a powerful spectroscopic technique in structural biology with many advantages. One of the greatest advantages is that virtually any sample in virtually any state may be studied by applying IR spectroscopy. It is a non-destructive method and it can monitor structural changes probed at less than a picosecond timescale. As a result of these advantages IR spectroscopy has become a well-established experimental technique for the analysis of the secondary structure of polypeptides and proteins under a wide variety of conditions.

In this article we will present a conformational study of a representative protein from the β-sheet family. We studied the lectin protein Concanavalin A (ConA). A ConA is composed of 237 amino acid residues, with molecular weight of 26.5 kDa. It has an ability to specifically bind on certain structures found in various sugars, glycoproteins, and glycolipids. This binding ability gives ConA some unusual biological properties: it takes part in denaturation of glycogen, dextrin, glycoproteins, and blood group substances [5–8]. Approximately 62% of its secondary structure is formed by two antiparallel β planes [9].

β-sheets are the prominent secondary structure template in globular proteins. Moreover, they are the building blocks of cross-β structures found in amyloidal plaque. The application of stepwise

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hydration on mainly β structured proteins thus provides a unique system to first explore the hydration mechanism with respect to the particular form of secondary structure and second it provides the ability to determine the limits of IR spectroscopy to detect small variations in protein conformation.

2. Materials and methods

A lyophilised powder of ConA was purchased from Sigma-Aldrich and was used without further purification. Protein water solution (10 mg/ml) was cast on ZnSe windows and dried out at 40 °C for one hour. Such prepared films should be thinner than 3 μm to avoid saturation of protein Amide I band [10]. The film thickness was measured by applying the standard interferometric method described elsewhere [11]. The measured density of the ConA cast film is 1.5 g/cm^3 determined by pycnometric method.

The stepwise hydration of protein was recorded in home-made transmission cell [12] (Fig. 1). The cell is similar to a transmission gas cell which has inserted a film holder. The cell is equipped with KBr windows. Temperature inside the cell (T = 25 °C) was precisely controlled by thermocouple. The relative water pressure was monitored by analysis of the intensities of water vapour rotational-vibrational bands [11,12]. Protein films have an atmosphere of controlled humidity created by bubbling of the dry nitrogen through H_2O. The recorded spectrum is a superposition of the spectrum of the surrounding atmosphere and correspondingly hydrated protein film. The maximum level of humidity of the reference spectrum (97–98%) of moisture nitrogen in the empty cell was measured by calibrated thermohygrometer (Oakton, Cole-Parmer Instruments Co.). This spectrum was used for subtraction of vapour bands and determination of the relative vapour pressure in the hydration experiment by assuming the linear relation between the intensity of vapour bands and relative pressure of water. The fully hydrated sample was slowly dried out by blowing dry nitrogen into the measuring cell. During the process of dehydration we were recording spectra of protein films in various states of hydration. The number of water molecules was determined by applying integral intensity of OH libration mode and film thickness [11].

All measurements of infrared spectra were performed on Perkin Elmer System 2000 FTIR and Bruker Vertex 80 spectrometer equipped with DTGS and MCT detectors. The ConA solution spectrum was recorded by Golden Gate diamond ATR cell thermostated at 25 °C. The displacement of the moving mirror of the interferometer was 0.25 cm that corresponds to a resolution of 4 cm^{-1}. Typically 32 interferograms were averaged. Absorbance spectra of protein solutions were recalculated from ATR spectra by applying the Kramers-Kronig transformation [13]. Post processing of the measured and calculated spectra (band fitting, Fourier deconvolution and second derivatives) was performed by the Grams software.

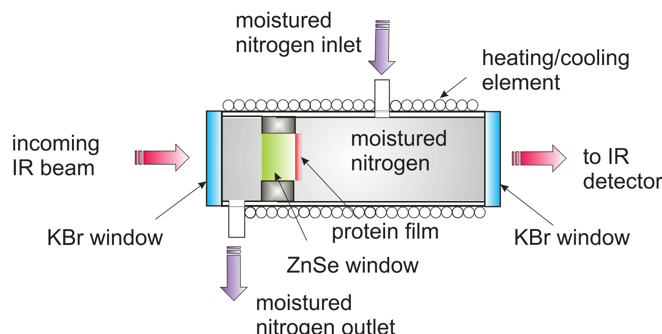

Fig. 1. Schematic presentation of infrared transmission hydration cell.

Thermogravimetric analysis was measured at T = 25 °C in the range between 0 and 90% of relative humidity by applying Surface Measurement Systems' Dynamic Vapour Sorption instrument.

3. Results and discussion

The most representative IR spectra of ConA are presented in Fig. 2 collected at different levels of hydration. The strongest protein band appears near 1635 cm^{-1} and belongs to Amide I vibration. The frequency and band shape of this mode are secondary structure dependent and thus frequently used as an indicator for protein structure. An Amide II band is less intensive and located near 1532 cm^{-1}. The origin of the former is mainly in backbone C=O vibration while in later mode dominates backbone N–H deformation mode. The next amide band, known as Amide III band, is located around 1234 cm^{-1}. In general, the Amide III bands appear in the spectra of proteins and peptides as moderately intensive band(s) in the region between 1320 cm^{-1} and 1240 cm^{-1}. This vibration arises mainly due to the N–H in-plane bending coupled to some other peptide modes (C–N stretching, C–C stretching, and C–O in-plane bending). The sensitivity of the Amide III band components for the change in conformation has been proven by numerous experimental and theoretical studies [14–17].

In a high frequency region of presented spectra are located the bands due to CH, CH_2 and CH_3 stretching vibrations of side-chains. These weak but sharp bands are located in region between 2980 cm^{-1} and 2870 cm^{-1}. Backbone NH stretching can be found at 3285 cm^{-1}. This band is in general overlapped with the band(s) of hydrating water, which in general appear(s) at slightly higher frequency. However, the separation is not trivial because OH stretching mode of water molecules which are involved in stronger H-bonds may be spread toward lower wavenumbers far below the location of NH stretching central peak.

A structural analysis was performed on the dry and fully hydrated films (Fig. 2a and c) as well as on Con A solution in water (Fig. 2d). The analysis was started on Con A in the form of fully hydrated film by determination of the secondary structure. We combine the band structure analysis on Amide I and Amide III bands to improve the reliability of band decomposition. In general, the application of band resolution enhancement techniques such as

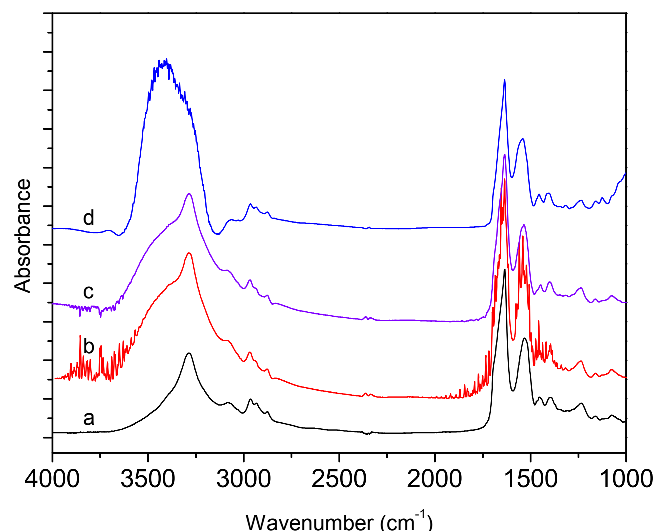

Fig. 2. Representative ConA spectra of a. dried protein film, b. fully hydrated protein film before subtraction of water vapour, c. fully hydrated film after subtraction of water vapour and d. ConA solution in water after subtraction of bulk water (thickness used in calculation is 1 μm). The protein film thickness is 0.42 μm.

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