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On the specificity of the amide VI band for the secondary structure of proteins

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ABSTRACT

In this work we analyzed the specificity of the amide VI band for different types of secondary structure elements in protein structures. This band involves the bending motion of the C=O group of the peptide chain that is typically observed in the spectral region from 590 to 490 cm^{-1}. The infrared absorbance spectra of a set of polypeptide model compounds of well known secondary structure was obtained at defined pH, including poly (L-lysine), poly (L-tyrosine), poly (L-alanine) and poly (L-histidine). In addition spectra of membrane proteins from the respiratory chain, namely the NADH:ubiquinone oxidoreductase, the cytochrome *c* oxidase and its Cu_A fragment, the cytochrome bc_1 complex, a Rieske-type protein and in addition myoglobin, have been comparatively investigated. The systematic analysis of the amide VI band of the polypeptides and the proteins allowed correlating the signal appearing at ~525 cm^{-1} to α-helical structures and signals at ~545 cm^{-1} to β-sheet contributions. Random coils have been found to contribute at ~535 cm^{-1} while the β-turns were observed at ~560 cm^{-1}.

1. Introduction

The knowledge on the structure-to-function relationship of a protein is crucial for the understanding of its reaction mechanism. Reversible modifications of the structure may be for example related to redox reactions, protein–protein, protein–ligand or protein–membrane interactions [1]. The misfolding and aggregation of proteins is believed to be a crucial step in some neurodegenerative disorders (Alzheimer's, Parkinson's and Huntington's diseases) [2]. *In vivo*, and due to a pathological misfolding, some proteins lose their native conformation and lead to abnormally large blocks of β-sheet type structures. These blocks can aggregate and turn into a clinically significant dysfunctional form of the protein. It was found that the main structural transition is a conformational transition between the α-helical and the β-sheet form. Techniques that allow studying the secondary structure of a protein are thus of great interest.

Infrared (IR) spectroscopy is a well established experimental technique for the analysis of the secondary structure of polypeptides and proteins. Molecular vibrations are sensitive to the hydrogen bonding environment and vibrations of the polypeptide backbone are typical for the structural organization of the peptide chain. Several vibrational modes in the mid and in far IR domain can be distinguished. In the mid IR range, the amide A (~3300 cm^{-1}), amide I, II and III (1700–1200 cm^{-1}) signals are easily identified. In the lower frequency domain, the amide IV, V, VI (800–500 cm^{-1}) and VII (below 250 cm^{-1}) are found. Beside the amide I band, the amide II, III and V bands were previously reported to be sensitive to the secondary structure modifications of proteins and peptides [3–5]. The amide I band, typically observed between 1700 and 1600 cm^{-1} includes the ν(C=O) vibration of the amide group in a hydrogen bonding dependent manner [4–8].

Oberg et al. [9] used the comparison between the circular dichroism and IR spectroscopy to obtain an accurate secondary structure analysis of a set of 50 proteins. It was described that circular dichroism is more accurate in the determination of the α-helical conformation than IR spectroscopy. On the other hand, the β-sheet conformation is clearer identified using the amide I and amide II bands positions in an IR spectroscopic approach, as compared to the circular dichroism [10]. Generally the use of the amide I band for the analysis of the secondary structures of proteins is well established.

The lower frequency region is little studied for proteins and as consequence the specificity of the amide VI band towards secondary structure of proteins was not analyzed yet; to our knowledge. The amide VI band was first described for N-methylacetamide [11]. In polyamide-containing polymers [12] (and references therein) the amide VI band appears at 580–590 cm^{-1} for polyesters and that the band position depends on the crystalline form [12]. Controversial descriptions concerning the vibrational modes of the amide IV and VI bands are available. Studies attributed the

Abbreviations: ATR, attenuated total reflection; DTGS, deuterated triglycine sulfate; FTIR, Fourier transform infrared; NADH, nicotinamide adenine dinucleotide; NDF, NADH dehydrogenase fragment; MES, 2-(N-morpholino)ethanesulfonic acid; DDM, n-dodecyl-β-maltosid; IR, infrared; PLK, poly (L-lysine); PLY, poly (L-tyrosine); PLA, poly (L-alanine); PLH, poly (L-histidine).

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vibrational modes to both, the out-of-plane bending motion of the C=O group of the amide function [11,12], as well as to the in-plane bending motion of the C=O group of the amide function [13]. Both, the amide IV and amide VI bands may thus arise from in-plane and out-of-plane motions of the CONH group in the peptide backbone and are expected in the region 700–500 cm^{-1}. According to the literature, these modes are generally not observed in Raman spectra [14].

Here we examine the amide VI band of a set of poly amino acids that show a well known structural arrangement. Poly (L-lysine) (PLK) is one of the most used model compounds in the field of protein aggregation since the transition that can be induced from the α-helical and the β-sheet form is the same that is discussed to take place during the protein aggregation process [10,15,16]. This transition is pH and temperature dependent. Venyaminov and Kalnin [10] showed that PLK has a random coil conformation at neutral pH. The changes to α-helix are observed around pH 11.2. Moreover, at this pH value, heating to 30–40 °C leads to the formation of the β-sheet conformation. The chain length is a major factor for the exact transition temperature; for chains shorter than 10 kDa, the transition does not take place [15]. Poly (L-tyrosine) (PLY) is also a model polypeptide studied in the field of protein aggregation and formation of β-pleated fibrils. As a reference for the pure α-helical form, poly (L-alanine) (PLA) is available. The previous vibrational analysis of the α-helical form was supported by the ab initio theoretical calculation, and the signals observed in the region 517–530 cm^{-1} were assigned to the amide IV band and not the amide VI band [17]. The study reveals the complexity of this amide band. The band arises from the mixture of multiple, coupled vibrational modes: the C=O in-plane bending motion, the C–C–N deformation, and C–C deformation. To avoid ambiguities the expression "amide VI" will be used to designate the band appearing in the region 590–490 cm^{-1} in this work.

In order to better understand the relation between the secondary structure and the amide VI position, data obtained for the poly amino acids with a well defined structure are compared to data for proteins from the respiratory chain where the secondary structure is mostly of a mixed character. The studied proteins are the NADH:ubiquinone reductase (respiratory complex I), the ubiquinone oxidoreductase (complex III), cytochrome *c* oxidase (complex IV) and the water soluble Rieske protein. The structure of most of these proteins is known [18–22].

2. Materials and methods

2.1. Sample preparation

Poly (L-lysine) 45 kDa, poly (L-histidine) 9.2 kDa and poly (L-alanine) 1076 Da were purchased from Sigma and deuterium oxide with a purity of 99.9% from Aldrich. The samples were prepared in an ice bath to avoid the spontaneous aggregation. The pH and the pD value was adjusted by adding small amounts of 0.1 M NaOH (NaOD) or HCl (DCl). Poly (L-tyrosine) (PLY) was purchased from Sigma and the ethanol (EtOH) of spectroscopic grade from Merck. For the IR analysis the solutions of PLY were prepared at a final concentration of 0.12 mM in EtOH. The protonation and deprotonation effects were studied in the presence of 0.1 M HCl and 0.1 M NaOH, respectively.

The NADH:ubiquinone oxidoreductase (complex I) from *Escherichia coli* was prepared as described [23]. Complex I was concentrated to a final concentration of 250 μM in 50 mM MES/NaOH, 50 mM NaCl, 0.1% DDM at pH 6. The NADH dehydrogenase fragment (NDF), the soluble part of the *E. coli* complex I containing the three subunits NuoE, NuoF and NuoG, was purified as described [24]. The soluble fragment with a final concentration of 200 μM was dissolved in 50 mM MES/NaOH, 50 mM NaCl at pH 6. Complex III and complex IV, both from *Paracoccus denitrificans*, were prepared as previously reported [25,26], in 50 mM Tris–HCl buffer at pH 8 containing 0.05% DDM. The concentrations of both samples are ~20 μM. The so-0called Rieske protein from *Thermus thermophilus* was prepared as previously reported [27] and dissolved in 20 mM phosphate buffer pH 7, to a final concentration of ~2 mM.

The Cu_A fragment was for spectroscopy as described in [28] following the protocol from [29] and measured in 10 mM phosphate buffer, pH 8, with a final protein concentration of about 1 mM. Equine myoglobin was purchased from Sigma–Aldrich lyophilized and studied in the same buffer.

2.2. Data collection

For the ATR-FTIR measurements, in the mid and in the far infrared 2 μL of the respective sample were used to obtain a film on the attenuated total reflection unit (ATR Harrick crystal, Diamond Prism). All samples were dried on the surface. Since complex I is a less stable protein, approximately 2–4 μL of the protein solution was dried under a gentle stream of nitrogen gas to form a thin film on the ATR silicon crystal (ATR Harrick crystal, Silicon Prism). Data in the mid and far infrared domain where measured with a Vertex 70 instrument from Bruker, the optics and the parameters where adapted for the different spectral ranges as follows: In the mid IR domain a detector mercury cadmium telluride (MCT) was used and the scan velocity was 20 kHz. Ten spectra with a resolution of 4 cm^{-1} (256 scans) were averaged for each sample. In the far IR domain a deuterated triglycine sulfate (dTGS) detector was used and the scan velocity was 2.5 kHz. For one sample five spectra with a resolution of 4 cm^{-1} (128 scans) were averaged. The instrument was purged with dry air in order to avoid contributions from humidity in the spectra. No baseline correction or smoothing procedure was applied to the absorption spectra shown here. During all measurements a gentle stream of argon protected the sample film to get in contact with humidity and to maintain the deuteration level.

2.3. Secondary structure determination

The secondary structure of the proteins was determined based on the amide I band found between 1700 and 1600 cm^{-1}. The analysis procedure is shown in detail in Fig. 1 for PLY. The position of each structural element was chosen from the second derivative spectra (Fig. 1B, full line). A shift of the position was allowed for fitting, leading to a lower error. Prior to curve fitting, a straight baseline, passing through the ordinates at 1700–1600 cm^{-1} (Fig. 1A) was subtracted. A least-squares iterative curve fitting was performed with Gaussian bands using the Peak Fit Analysis Program (SeaSolve, MA, USA). Each band obtained (Fig. 1 A) from the fitting procedure was assigned to a secondary structure element according to Goormaghtigh et al. [13] The areas of all bands assigned to a given secondary structure were then summed up and divided by the total area in order to obtain the contribution of each element. The assumption was made that the absorption coefficients were equal for all the secondary structures [30–32,5]. Differences between the absorptions coefficients of the secondary structural elements have been described in literature [33] however, we note that most authors work with an equal absorption coefficient inducing a systematic error.

The amide VI band arises in the spectral range from 590 to 490 cm^{-1}. In the given measurements it seems to be about 3 times less intense than the amide I band. We note, however, that not only a precise absorption coefficient is not valid for the measurements in films, also the penetration depth of the evanescent wave