Structural Changes of PLL by Electric Field Stimulation: A FTIR ATR Modulation Spectroscopic Study

M. Schwarzott, D. Baurecht, U.P. Fringeli
Institute of Physical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Introduction

The investigation of the effects of an electrical field on biological molecules (e.g., biomembranes or proteins) by infrared spectroscopy is a final goal of our group. The presented experiments are one of the first steps on this way. We choose Poly-L-Lysine (PLL) as a model peptide because there are former experiences with a temperature modulation Fourier transform infrared (FTIR) attenuated total reflection (ATR) spectroscopic study[1]. It is well known that this polypeptide exhibits secondary structure conversions depending on various thermodynamic parameters[2]. Because of the internal dipole moments of PLL which originates for instance from the intrinsic dipole of the α-helix or the different charged ε-amino group at varying pD-values, there should be the possibility of an influence by an electrical field.

Materials and Methods

1 mg/mL PLL-HBr (Fluka; MW.: 5000 - 10000) was dissolved in D_2O (Aldrich; 99,9 atom %D) and the solution was adjusted by NaOD (Fluka; ~ 40% in D_2O) to a pD value of 10,6. The sample was filled in the flowthrough cell (schematically shown in Fig.1) by a syringe. Both sides of the "sandwich"-like cell are used, leading to a mean number of active internal reflections N=33 at an angle of light incidence θ=45°. The cell was thermostated at a temperature ot 25°C. Infrared measurments were performed on a Bruker IFS66 FTIR spectrometer with a resolution of 4 cm^{-1}. A function generator applied a periodically rectangular electrical potential to the Ge-IRE (Fig. 1) from + 50mV to -1V. Time resolved modulation spectra were recorded followed by a digital phase sensitive detection (PSD).

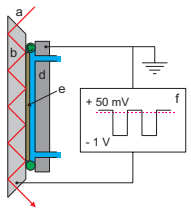

Fig. 1: Scheme of the experimental set-up. *a) polarized infrared light, totally reflected b) Germanium crystal, used as trapezoid internal reflection element (IRE) and first electrode c) VITON O-ring, sealing and isolating spacer between Ge and the flowthrough cell d) One part of the metal flowthrough cell, arranged with the IRE as "sandwich" and serving as second, grounded electrode. e) from solution adsorbed PLL-layer on the Ge surface. f) function generator, applying a rectangular electrical potential between +50 mV and -1 V*

Demodulation by Digital Phase Sensitive Detection (PSD)

A time dependent modulation measurement requires a periodical external stimulation, as in this case the change of the elctrical potential. Within a stimulation period T time resolved spectra were recorded to get the absorption $A(\tilde{\nu},t)$ as a funktion of wavenumber and time. Every periodical function can be expressed as a Fourier series in the form

$$A(\tilde{\nu},t) = A_0(\tilde{\nu}) + \sum_{k=1}^{\infty} A_k(\tilde{\nu}) \sin\left[k\omega t + \varphi_k(\tilde{\nu})\right]$$

where $A_0(\tilde{\nu})$ is the mean absorption during a period (a time-independent DC term), the $A_k(\tilde{\nu})$ are the power amplitudes and the $\varphi_k(\tilde{\nu})$ are the phase lags refered to the stimulation period. The index k indicates the order of the harmonic components with the fundamental frequency $\omega = 2\pi/T$ at k = 1. To evaluate these power amplitudes and phase lags by PSD, a additional phase lag ϕ_k^{PSD} is introduced. The demodulation is described by

$$A_k^{\phi_k^{PSD}}(\tilde{\nu}) = \frac{2}{T}\int_0^T A(\tilde{\nu},t)\sin(k\omega t + \phi_k^{PSD})dt \quad (k = 1,2,\ldots)$$

The chosen phase lag determine the shape of the phase resolved absorption spectra $A_k^{\phi_k^{PSD}}(\tilde{\nu})$ and they are related to the Fourier power amplitudes by

$$A_k^{\phi_k^{PSD}}(\tilde{\nu}) = A_k(\tilde{\nu})\cos\left[\varphi_k(\tilde{\nu}) - \phi_k^{PSD}\right]$$

If the PSD phase angle equals the Fourier phase lag, one obtains the power amplitudes $A_k(\tilde{\nu})$ .Amplitudes becomes zero when the difference between $\varphi_k(\tilde{\nu})$ and ϕ_k^{PSD} becomes $\pm 90°$. The use of a vector-PSD[3] of the whole spectra after data sampling allows the computation of the modulation spectra within any interesting range of the PSD phase lags.

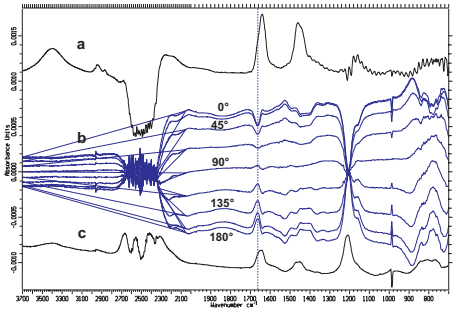

Fig. 2: Parallel polarized stationary spectrum and phase resolved spectra at the fundamental frequency (1ω). *The solution of 1mg PLL /mL at pD = 10,6 was placed into the flowthrough cell and stayed unchangend during the measurements. Spectra (a) and (c) are multiplied by a faktor of 0,02. (a) Spectrum of the adsorbed PLL-layer before modulation experiment. (b) Phase resolved spectra calculated by digital PSD at phase settings from 0° to 180° with an equidistant phase resolution of 22,5°. A rectangular electrical field stimulation with a period T=20min was applied. A PSD phase angle of 0° means in phase with the switching of the electrical potential from -1V to +50mV. The shifts of the baselines and the most prominent band at 1205cm^{-1} originate from the influence of the different electrical polarity to the Ge crystal and to the bulk media. The dashed line at 1662cm^{-1} marks the dominant response of the Amide I´ band of PLL due to modulation. There is an evident shift of the absorption maxima compared to the stationary spectra (a and c) showing the effect on the secondary structure of the polypeptide. (c) Difference of stationary spectra before and after the modulation experiments. The increase of the PLL absorption is explained by a further adsorption during the time of measurement of the previous modulations which has taken about 20 hours.*

Results and Discussion

The Poly-L-Lysine at pD=10,6 adsorbs spontaneously on the Ge surface when placed into the flowthrough cell (Fig.1). The shape of the Amide I`with its maximum absorption at 1641cm^{-1} is typical for PLL with a structural mixture of mainly α-helix and random components[1,2] (Fig.2). At this pD-value an almost uncharged peptide is expected because of the known helix to β-sheet conversion of solved PLL with MW.:30000-70000 in the pD range of 10,5 -11,0 (unpublished data). However, the $\delta_{as}(ND_3^+)$ can be assigned in the phase resolved spectra (Fig.3) whereas it is impossible to do so in the stationary difference spectra.

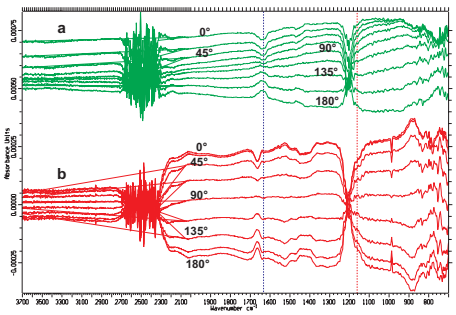

Fig.3: Parallel polarized phase resolved spectra at the first (a) and second harmonic (b) frequency (2ω and 3ω) of adsorbed PLL at pD = 10,6. *Both are demodulated with a phase resolution of 22,5° and led from 0° to 180°. The spectra derive from the same modulation experiment as described in Fig.2 with a stimulation period of 20min. By comparing the absorptions of the fundamental (Fig.2)and second harmonic one finds the same qualitative behaviour except the smaller amplitudes in the overtone spectra. On the other hand, in the Amide I´ region the response at the first harmonic differs unambiguously from that which can be noticed in the fundamental or 2nd harmonic (blue dashed line). In curve fitting analysis this component at 1638cm^{-1} (a) is typically assigned as a α-helix[1]. The band at 1160cm^{-1} seen in both spectra series (a and b)] results from the antisymmetric ND_3^+-bending ($\delta_{as}(ND_3^+)$, red dashed line). These small changes with an amplitude of about 0,02mAU shows the sensitivity of the modulation spectroscopy.*

This improvement of the signal to noise ratio, is one advantage of the modulation spectroscopy. If the period of the external stimulation is in the range of the kinetic time constants, the dynamic processes can be analysed. In the case of PLL, periods of T=10min to T=40min were used. Although the signals are very small, the mentioned condition is met and the stimulation of structural changes occurs which can be observed in the Amide I´ and Amide II´ region (Fig.2-4). The strong electrical potential and therefore the surface charge density at the Ge/PLL surface is modulated by a strictly rectangular signal. that possesses only Fourier components with odd frequencies. If the PLL would act as a linear system (that means e.g. all first order or pseudo first order kinetics) there were no answer in the first harmonic. As shown in Fig.3, the polypeptide respond with frequency 2ω indicates a non linear folding step. ATR spectroscopy with polarized light ofefrs an opportunity of oriented measurement[4]. The experiments were performed with parallel as well as perpendicular polarized IR-light but no dichroic effects are noticed. Decomposing of strongly overlapping IR bands (e.g. Amide I´) is a preconditon to determine the dependence of structure components of the external parameter. A method to correlate phase resolved spectra[1] (e.g. curve fitting, 2D-IR correlation analysis) can assign components from different spectral regions to each another demonstrated with the $\delta_{as}(ND_3^+)$ and $\delta_s(ND_3^+)$ bands in Fig.4.
These first experiments with an external electrical field applied on the model peptide PLL show the advantages of modulation spectroscopy and hoping to get new insight in the effects of electrical fields on protein structure.

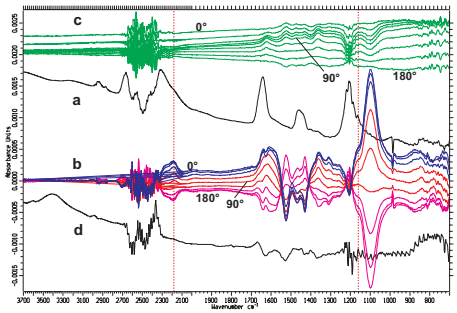

Fig. 4: Parallel polarized stationary spectrum and phase resolved spectra at the fundamental (ω) and first harmonic (2ω) frequency of the PLL-layer in contact with D_2O. *Spectrum (a) is reduced by a factor of 0,02 and (d) by 0,1. (a) Spectra of PLL after changing the original bulk phase at pD = 10,6 by D_2O and before the modulation experiment has started. No loss of the adsorbed PLL-film during this washing process could be noticed. The electrical potential at stationary measurement was set to 0V. (b and c) Phase resolved spectra calculated by digital PSD at phase settings from 0° to 180° (phase resolution = 22,5°). The rectangular electrical field stimulation occurs with a period of T = 10min. The change of the solvent causes different effects of the baseline and in the absorption of the D_2O bending ($\delta(D_2O)$ at 1205cm^{-1} compared to spectra (b) in Fig.2. The strong bands at 1530cm^{-1} and 1100cm^{-1} couldn´t assigned up to this moment. Because of the high intensity we suppose a solvent effect that should be enlighted by an H-D change experiment. In the Amide I´(1690cm^{-1}- 1610cm^{-1}) and Amide II´(1490cm^{-1}- 1420cm^{-1}) region appear new components refered to Fig.2-3, e.g. a broad band at 1620cm^{-1} in the fundamental and 1st harmonic. The changes of the residues of PLL are noticeable by the weak modulations of the CH_2 stretching bands around 2900cm^{-1}. The correlation of bands is shown between the antisymmetric (1160cm^{-1}) and symmetric (1180cm^{-1}) bend of ND_3^+ (both marked with red dashed lines). Their modulation amplitudes vanished at the same PSD phase lag at about 112,5°. (d) Difference spectrum before and after modulation measurements. No additional adsorbtion occurs instead an increase in the Amide I´ band at 1665cm^{-1} is observed correlated with a decrease at 1630cm^{-1}.*

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Correspondence: Michael Schwarzott < michael.schwarzott@univie.ac,at >