THz and IR Spectroscopy of Molecular Systems That Simulate Function-Related Structural Changes of Proteins

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Abstract. The activity of enzymes in organic solvents substantially increases in the presence of crown ethers. Tris(hydroxymethyl)aminomethane (tris) is chosen as a model compound to simulate the interaction of surface amino groups of proteins with crown ether. The methods of FTIR and time-domain THz spectroscopy are used to study the interaction of tris with 18-crown-6. The THz spectra of the complexes are measured for the first time.

Keywords: THz spectroscopy, FTIR spectroscopy, tris, crown

1. Introduction

The analysis of the protein structure is an important problem in the study of biological molecules, since the functional activity of protein is related to structural changes. It is known that the enzymatic activity in organic solvents (e.g., of chymotrypsin) sharply increases in the presence of crown ethers [1, 2]. One of the possible reasons is the interaction of crown-ether molecules with the surface amino groups of protein. Note that the vibrational bands of the amino groups are strongly overlapped with the protein amide bands, and the methods of IR and Raman spectroscopy cannot be directly employed in the corresponding study. However, the spectroscopic methods can be used to investigate the interaction of amino groups with crown ethers in model chemical systems. One of the model compounds is tris(hydroxymethyl)aminomethane (tris) ($(\text{HOCH}_2)_3\text{C-NH}_2$ [3], and 18-crown-6 ($\text{C}_{12}\text{H}_{24}\text{O}_6$) is the crown ether in our experiments. The purpose of this work is the detection of the spectral changes in the IR and THz spectra due to the interaction of amino group with crown ether.

2. Experimental

We employ tris and 18-crown-6 from REAHIM. Tris and its complexes with 18-crown-6 at several relative concentrations are prepared as powders using the 20-hour-long lyophilization of solutions with

different pH. Hydrochloric acid is used for the adjustment of pH (pH10, pH8.3, pH7.8, pH3). The samples represent pressed tablets with a diameter of 5 mm and a thickness of about 300 μm . For the measurement of the IR spectra, we employ a Nicolet 6700 FTIR spectrometer interfaced with a Smart Orbit ATR unit. The THz setup is described in [4].

3. Results and Discussion

In the wavenumber interval 1500–1700 cm^{-1} , the IR spectrum of tris at pH10 exhibits a single band peaked at about 1586 cm^{-1} , whereas the spectrum of tris at pH3 exhibits two bands peaked at 1550 and 1628 cm^{-1} . The three bands are detected in the spectra of tris at intermediate pH (pH7.8 and pH8.3).

The IR spectrum of the complex of tris with 18-crown-6 at a relative molar concentration of 1 : 1 and pH10 can be well fitted using the spectra of components and polynomial background [5]. The measured spectrum of the complex and the fitting curve are very close to each other except for minor spectral differences in the interval 1050–1200 cm^{-1} . However, the spectrum of the same complex at pH3 significantly differs from the sum of the spectra of components in the fingerprint range. Note that the two bands of the protonated tris (pH3) peaked at 1550 and 1628 cm^{-1} are changed by a single band peaked at 1587 cm^{-1} with a shoulder at 1600 cm^{-1} . These results indicate that the protonated tris is involved in significantly stronger interaction with 18-crown-6.

Figure 1(a) demonstrates the THz spectra of tris at several pH. It is seen that the spectrum of the protonated tris (pH3) substantially differs from the remaining spectra, since it does not exhibit developed bands peaked at 1.51, 1.73, 2.11, and 2.30 THz. Figure 1(b) shows the spectra of tris at pH10, 18-crown-6, and the complexes at relative molar concentrations (tris/crown) of 1 : 1 and 1 : 10 and pH10. Note that the spectra of the complexes at relative molar concentrations of 1 : 2 and 1 : 5 (not shown) slightly differ from the spectrum of the complex at 1 : 10. The complex formation leads to significantly lower intensities of the bands peaked at 1.52, 1.75, and 2.30 THz. When the relative molar concentration of 18-crown-6 increases to 10, the above bands of the pure tris almost vanish and the broad band at 1.65 THz can be assigned to the free 18-crown-6. Nevertheless, the band peaked at 2.11 THz survives in the spectra of complexes.

Figure 1(c) demonstrates the spectra of tris at pH3, 18-crown-6, and the complexes at relative molar concentrations of 1 : 1 and 1 : 10. As distinct from the results at pH10, the spectra of the complexes at intermediate relative concentrations of 1 : 2 and 1 : 5 (not shown) differ from the spectra at 1 : 1 and 1 : 10. Note also significant spectral changes due to the interaction with the crown ether. The strongest spectral changes correspond to the transition from the spectrum of the protonated tris to the spectrum of the equimolar complex. The latter exhibits three developed bands. The first (broadest) band with a width of about 0.4 THz is peaked at about 1.30 THz. Two remaining (narrower) bands are peaked at 2.10 and 2.31 THz. The results indicate structural changes of the protonated tris caused by the interaction with 18-crown-6. It is seen that an increase in the relative concentration of the crown ether leads to a decrease in the intensity of the band peaked at 1.30 THz. Apparently, the band is not assigned to 18-crown-6. We assume that the spectra of complexes with high relative concentrations of the crown ether can be represented as the sums of the spectra of the crown ether and the tris complex with the crown ether at a relative concentration of 1 : 1. Based on such an assumption, we assume that the broad band can be assigned to the protonated tris that is structurally modified owing to the interaction with the crown ether. Thus, the spectral data indicate two transformations of the protonated tris related to (i) the transition

Figure 1: THz spectra of (a) tris at (solid line) pH10, (triangles) pH7.8, and (squares) pH3; (b) tris-18-crown-6 complexes at pH10 and relative molar concentrations of (circles) 1 : 0, (squares) 1 : 1, (triangles) 1 : 10, and (solid line) 0 : 1; (c) tris-18-crown-6 complexes at pH3 and relative molar concentrations of (circles) 1 : 0, (squares) 1 : 1, (triangles) 1 : 10, and (solid line) 0 : 1.

from the unbound molecule to the equimolar complex and (ii) an increase in the relative concentration of the crown ether.

Note that the spectra of the complexes of protonated tris at relative concentrations of 1 : 5 and 1 : 10 are similar to the spectrum of the equimolar complex of tris at pH10. This circumstance can be an indication of the deprotonation of the protonated tris in the complexes with high relative concentrations of the crown ether.

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