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Vibrational circular dichroism of amylose carbamate: structure and solvent-induced conformational changes

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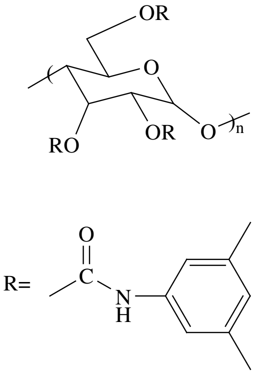
ABSTRACT

The conformational changes of amylose tris-(dimethylphenylcarbamate) (ADMPC) in the presence of polar solvents were investigated by VCD spectroscopy for the first time. The conformation of ADMPC was monitored using this technique in the solid film state and in the presence of different concentrations of alcohols, such as ethanol, *n*-propanol, and isopropanol in mixtures with hexane. The VCD results revealed that in the presence of these solvents, the polymer undergoes a drastic conformational change.

1. Introduction

Amylose is a polysaccharide made up of about 100 to several thousand D-glucose units joined together by α(1→4) glycosidic bonds and is one of the components of starches (along with amylopectins).[1] While amylose is a linear polysaccharide, amylopectins are mixtures of branched polysaccharides. The use of amylose and amylopectins in the food industry is limited by their low stability at lower temperatures and upon storage.[2] Due to the geometry of their α(1→4) linkage, the main chains of the D-glucose units in amylose tend to assume a coiled helical conformation along with the sugar, which give amylose a certain chirality.[3] Given its chirality, amylose has been widely used as a support for chiral discrimination.[4] Chemical modification of the hydroxyl functional groups improved the stability of amylose and its use in the food industry as well as in fields such as chiral separation.[2,5] Among all the chemical modifications, amylose tris-(3,5-dimethylphenylcarbamate) (ADMPC) is one of the most promising and versatile derivatives for the application to the separation of enantiomers[6,7] (structure shown in Scheme 1). Its helical structure forms chiral cavities, where enantiomers can be included, forming complexes which differ in their stability.

ADMPC is one of the most versatile chiral stationary phases (CSPs) able to separate a wide variety of enantiomers. During the separation process, however, CSPs undergo subtle conformational changes, which in turn affect both the performance of the separation and the enantiorecognition. These conformational changes have been cited in the literature and can be induced by the choice of a polar modifier, such as alcohols in mixtures with non-polar solvents (i.e., hexane), as well as by temperature.[8–10]



Scheme 1. Structure of ADMPC.

Vibrational circular dichroism (VCD) measures the difference in absorbance of a chiral molecule upon interacting with a left versus right circularly polarized light. As such these interactions occur in the mid-IR region and provide rich molecular level information on the functional groups that are directly affected by the chirality of the molecules. As a consequence, VCD spectroscopy has been extensively applied to determine the configuration and the conformation of various chiral molecules.

Herein we report, for the first time, the VCD spectra of ADMPC in both solution and dry film states. VCD has been widely used to study the structure of biopolymers such as proteins and peptides.[11,12] The use of films to study proteins by VCD has been previously reported.[13] Here, we use VCD to study the conformation of ADMPC in both solution and film states. The advantage of this method (compared to other techniques such as solid state NMR, X-ray, or IR) is the possibility of exploring the chiral environment of this biopolymer at the functional group level.

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2. Results and discussion

2.1. VCD of ADMPC

The structure of ADMPC was first studied by VCD in solution state, as shown in Figure 1. The band assignments are listed in Table 1. The VCD measurements were carried out on a Chiral *IR* spectrometer (BioTools, Inc., FL, USA) using the dual polarization modulation method.[14]

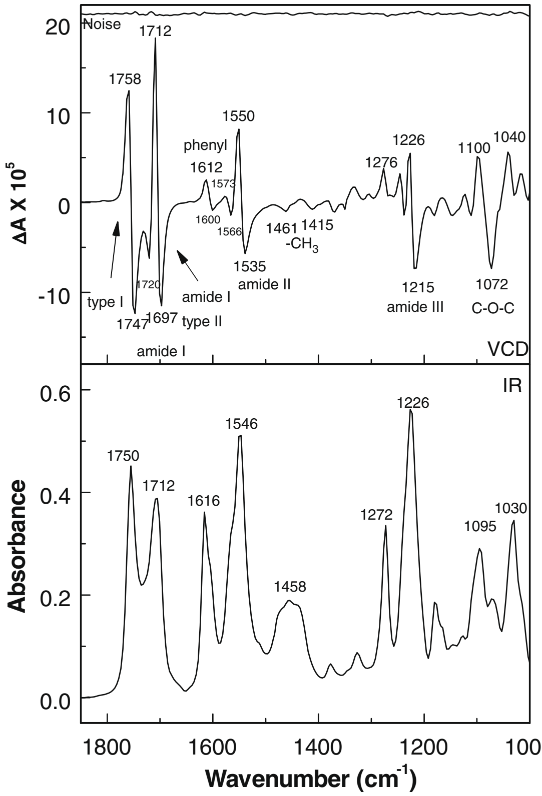

Figure 1. VCD and IR spectra of ADMPC in CD_2Cl_2 solution: The VCD spectra of ADMPC in CD_2Cl_2 solution were collected for 12 h with 200 μm pathlength at the concentration of ~15 mg/ml. VCD and IR spectra were solvent subtracted. Noise level was offset for clarity.

From the VCD spectra of ADMPC, it can be observed that the amide I bands (C=O coupled with –NH) present two negative couplets (+−, +−) with the corresponding maxima and minima at 1758, 1747 cm^{-1} and 1712, 1697 cm^{-1}, respectively. Such negative couplets imply that ADMPC may adopt a secondary structure such as a left-handed helix.[15] This type of feature is consistent with the previous X-ray results which suggest that ADMPC has a left-handed 4/1 helical conformation.[15] Similar results were obtained using solution and solid state NMR.[7,9] Similar to IR spectra,[9] VCD also indicates the existence of two types of C=O groups: type I and type II (Table 1). These two types of C=O correspond to weakly (interchains) and strongly bonded hydrogen bond (intrachain) to the NH groups, respectively.[9] The existence of two types of carbonyl groups is clearly shown in the IR spectra as well, with two IR bands at 1750 and 1712 cm^{-1}, respectively. The IR results are consistent with a previous ATR-FTIR study.[9] The VCD arising from the C=C of the aromatic group shows a positive band at 1612 cm^{-1} and a small negative band at 1600 cm^{-1}, corresponding to the IR band located at 1616 cm^{-1}. Similar to amide I, the amide II bands also exhibit two negative couplets, located at 1573, 1566 cm^{-1} and 1550, 1535 cm^{-1}, respectively. These couplets of amide II are due to the hydrogen bond interaction between the amidic NH and the two types of C=O exhibited by the two couplets of amide I. On the other hand, the type II of amide II (low frequency) shows much larger intensities than type I of amide II (high frequency), while the two types of amide I bands show similar intensities.

Table 1
Band assignment for ADMPC: (+) denotes positive VCD band, (−) denotes negative VCD band

Band (cm^{-1})	Vibrational modes
~1758 (+) ~1747(−)	Weakly H-bonded amide I (type I)
~1712 (+) ~1697 (−)	Strongly H-bonded amide I (type II)
~1616 (+) ~1600 (−)	Aromatic C=C
~1573 (+) ~1566 (−)	Amide II: type I
~1550 (+) ~1535 (−)	Amide II: type II
~1461 (−) ~1431 (+) ~1415 (−)	CH_3 bending linked to phenyl group
~1276 (+)	CH bending (from glucose)
~1226 (+) ~1215 (−)	C–N stretching coupled with C=O stretching (amide III)
~1100 (+) ~1070 (−) ~1040 (+)	C–O–C stretching

Interestingly, the VCD of glycosidic bonds (C–O–C) exhibits relatively strong peaks at 1100 cm^{-1}, 1072 cm^{-1}, and 1040 cm^{-1} with a (+, −, +) feature. These results are consistent with previous VCD studies on carbohydrates.[13] Their results suggest that the α-linkage of carbohydrates gives rise to strong VCD features for the glycosidic bond with stretching vibration between 900 and 1200 cm^{-1}.

The VCD of ADMPC was further studied in the dry film state. The dry film of ADMPC was prepared by casting an aliquot of 200 μl of ADMPC solution (~10 mg/ml in CH_2Cl_2) on a BaF_2 window. The window was placed in a fume hood with constant air flow at room temperature to dry for approximately 20 min prior to the VCD measurements.

Comparison of ADMPC in solution with ADMPC of dry film (Fig. 2) shows that most of the VCD and IR features from the film are preserved in solution. The IR spectra of the film show broader IR bands. The corresponding VCD bands of the film show similar broadening with smaller intensities.

These broader bands and smaller VCD intensities observed throughout the whole spectroscopic region ranging from amide I to C–O–C stretching regions (1700–1000 cm^{-1}) in the film state, indicate less regularity/organization of the polymer in the film state, which is consistent with XRD and NMR studies.[9] Moreover, VCD presents some additional bands in solution compared to the dry film. One of the most obvious features is the negative VCD band at 1720 cm^{-1} (Fig. 1), which cannot be observed in film. This additional feature was also observed in the VCD spectra of film in the presence of high concentrations of alcohol (Figs. 3–5, discussed in the following section).

2.2. Influence of alcohols on VCD of ADMPC

In order to study the influence of alcohol concentration on the VCD spectra of ADMPC, a number of solvent mixtures containing alcohols and hexane were prepared. The content of the polar component in the solvent mixture was varied from 1% to 20%. Hexane was selected for the solvent mixture because hexane has no influence on the structure of amylose carbamate.[10] It is important to note that the film was not soluble in any of the concentrations of

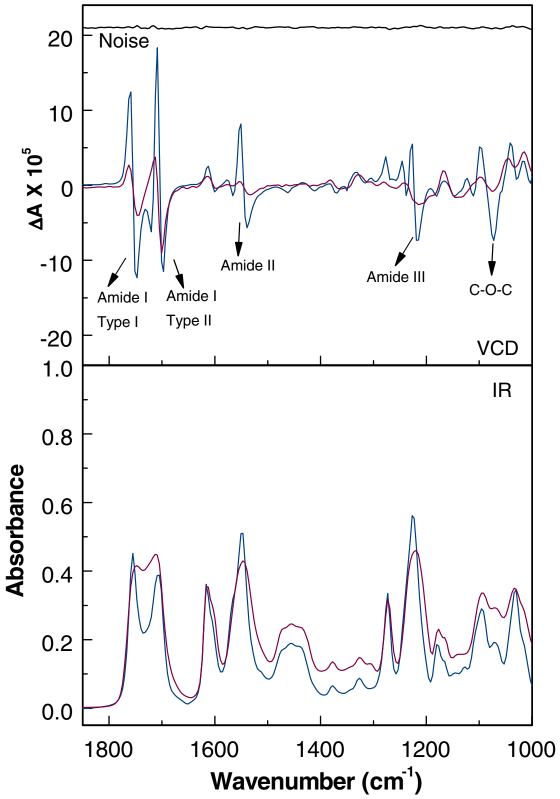

Figure 2. Comparison of VCD and IR spectra of ADMPC in solution (blue trace) with dry film (red trace). The solution spectra are the same as Figure 1. Dry film spectra were recorded for 3 h with 8 cm^{-1} resolution. VCD and IR spectra were subtracted by corresponding blank window for dry film. Noise level was offset for clarity.

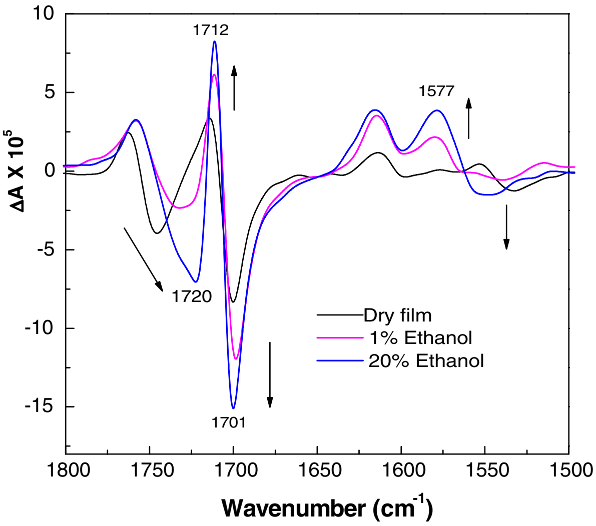

Figure 3. VCD of ADMPC dry film in the absence and presence of hexane/ethanol mixtures. All the VCD spectra were measured for 3 h at 8 cm^{-1} resolution followed by subtracting the VCD of a blank window measured at the same conditions for baseline correction.

hexane–alcohol. As a result the spectra show the behavior of the amylose carbamate at its interface with the solvent mixture.

For the measurements of the dry film in the presence of alcohol solvents, the prepared dry film was subsequently covered with another BaF_2 window of the same size through a 12 μm Teflon spacer. Approximately 100 μl of a hexane–alcohol mixture was injected between the two BaF_2 windows. The solvent and the film were equilibrated for 20 min prior to the VCD measurements.

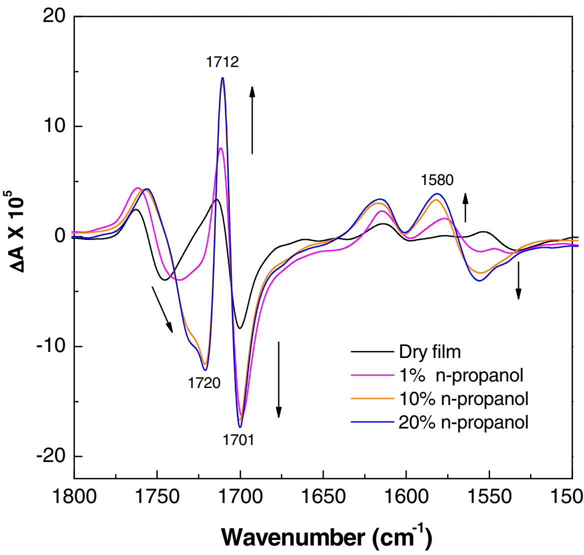

Figure 4. VCD of ADMPC dry film in the absence and presence of hexane/*n*-propanol (*n*-prop) mixtures. Experimental conditions are the same as Figure 3.

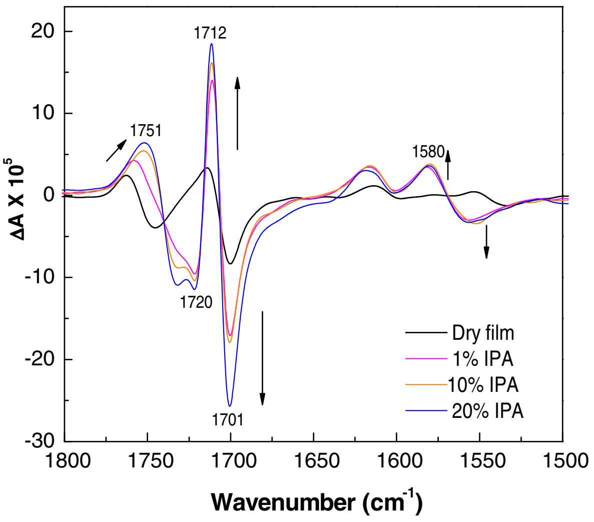

Figure 5. VCD of ADMPC dry film in the absence and presence of hexane/isopropanol (IPA) mixtures. Experimental conditions are the same as Figure 3.

Figures 3 and 4 show the behavior of ADMPC at the interface with a mixture of hexane/ethanol and hexane/*n*-propanol, respectively. When increasing the concentration of *n*-alcohol in hexane, the VCD bands showed an enhanced intensity, which indicates a higher-order structural organization in the presence of *n*-alcohols compared to the dry film. The type I C=O had a red shift in the presence of *n*-alcohols relative to the dry film; type II did not exhibit any shift but rather demonstrated an increase in the VCD intensity of the bands. In contrast, amide II had a blue shift in the presence of all the alcohols studied, compared to the dry film. Increasing the amount of alcohol in the mixture increases the shift and the band intensity. Such a phenomenon can be explained by the ability of the amidic NH to hydrogen bond in the dry film with a neighboring C=O. The addition of alcohol leads to an increase in the polarity of the environment. As a consequence, it decreases the strength of the hydrogen bonding between the NH and C=O at the expense of the formation of new hydrogen bonding between the type I C=O and the alcohol molecules. At the same time, increasing the concentration of *n*-alcohol led to the appearance of an additional negative VCD band in the ADMPC spectra, which was also observed in deuterated methylene chloride solution (Fig. 1). The exact position of this band is different from that observed in the CD_2Cl_2 solution due to the interaction of the film with the alcohol solvent. The appearance of this band ($\sim$1730 cm^{-1}) can be seen more clearly during the experiments using isopropanol (Fig. 5).

The VCD of the amide II bands of the film and the solution state present a sequence of a small positive and small negative bands, followed by a large positive and large negative band. The intensity of these bands is higher in solution than in dry film (Fig. 1). The band features, however, are preserved, regardless of the state of the polymer (dry film or solution). These findings are consistent with the existence of two types of NH groups, which are similar to those described in the IR experiments.[9] This shows that the hydrogen bond strengths are broadly distributed between the C═O and N–H groups of the biopolymer. In addition, in the presence of *n*-alcohols the VCD band at $\sim$1554 cm^{-1} starts decreasing in intensity (compared to the bands in the dry film), eventually switching sign from positive to negative upon increasing the concentration of *n*-alcohol. The negative VCD band at $\sim$1531 cm^{-1} of dry film becomes a shoulder upon increasing the concentration of *n*-alcohols. It initially shows a red shift; further increasing the *n*-alcohol concentration results in the broadening of the negative VCD band. This behavior indicates that the ADMPC forms hydrogen bonds with the alcohols, leading to a conformational change. In the presence of isopropanol (Fig. 5), this change from positive to negative of the band at 1554 cm^{-1} is more drastic, being independent of the concentration of isopropanol. To the best of our knowledge this is the first direct evidence of a major conformational change of the ADMPC induced by the presence of alcohols.

These results can be explained if we consider the different behavior between linear alcohols and branched alcohols.[8,16] It has been shown by IR spectroscopy that mixtures of linear alcohols in non-polar solvents undergo OH···OH self-association to form cyclic tetramers.[17] At concentrations near 4% the volume of the relative amount of tetramer to monomer is $\sim$50%. At the concentrations of the linear alcohols used in this study it is expected that ethanol and *n*-propanol will exist in an equilibrium state between the monomer and the aggregate. Therefore, the interaction of these alcohols can proceed to different extents. In contrast isopropanol exists mainly as monomers and as a consequence it will interact with the amylose carbamate more efficiently.

3. Conclusions

This is the first report on VCD of ADMPC which shows conformational changes induced by alcohols at the interface with the polymer. The experiments revealed that the polymer presents a more organized structure in solution than in dry film. In the presence of *n*-alcohols, the amide I bands split into two additional negative bands, indicating that not all of the C═O groups are equivalent in their interactions with the alcohols. The changes observed in VCD spectra arising from the interactions between alcohols and ADMPC in the film state reported here offer promise for the use of VCD as a new spectroscopic tool to understand differences in interactions between wide classes of chiral molecules.

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