FTIR studies of hydrogen bonding between \( \alpha, \beta \)-unsaturated esters and alcohols\(^1\)

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Abstract

The enthalpy (and entropy) of hydrogen bond formation has been measured between the ester carbonyl groups of the two \( \alpha, \beta \)-unsaturated esters thiencylacryloyl (TAOMe) and 5-methylthienylacryloyl (5MeTAOMe) methyl ester and the hydrogen bond donors ethanol, phenol and 3,5-dichlorophenol in CCl\(_4\). For the esters, the hydrogen bonding strengths were measured by quantitating the amount of bound and unbound donor, using the O-H stretching region, as a function of temperature and applying the van't Hoff equation. The decrease in \( \nu_{C=O} \) of the ester carbonyl group upon hydrogen bond formation (\( \Delta \nu_{C=O} \)) has also been measured and correlated with the enthalpy of hydrogen bond formation. A linear correlation is observed between the enthalpy of hydrogen bond formation \( -\Delta H \) and \( \Delta \nu_{C=O} \), with \( -\Delta H = 1.36 \Delta \nu_{C=O} - 16.1 \), where \( \Delta H \) is measured in kJ mol\(^{-1}\) and \( \Delta \nu \) in cm\(^{-1}\). Comparison with data for other carbonyl acceptor compounds indicates that the carbonyl group of the above \( \alpha, \beta \)-unsaturated esters is more readily polarized than the carbonyl group of saturated esters or ketones. The quantitative relationship between \( -\Delta H \) and \( \Delta \nu_{C=O} \) derived here has been used to determine the change in the enthalpy of hydrogen bond formation between substrate and enzyme groups in a series of acylserine proteases.

Keywords: Hydrogen bonding; Carbonyl; Unsaturated ester; Alcohol

1. Introduction

The vibrational spectrum of an enzyme-bound substrate contains detailed information concerning the structure of the bound substrate [1,2]. Potentially, the vibrational data can yield detailed quantitative information, such as the length of individual substrate bonds and the energy of individual enzyme–substrate interactions. In order to translate vibrational frequency changes into interaction energies, detailed studies on simple model compounds are required. The present study sets out to establish a relationship between carbonyl shifts and hydrogen bonding strengths that can be used to measure the strengths of hydrogen bonding interactions in the active sites of enzymes.

Both Raman and FTIR spectrosopies have
been used to probe the environment of substrate carbonyl groups in enzyme active sites. These techniques have been used to detect polarization of substrate carbonyl groups by the enzyme’s active site in a variety of systems, including triose phosphate isomerase [3,4], citrate synthase [5], phospholipase A₂ [6], chloramphenicol acetyltransferase [7], lactate dehydrogenase [8], and ketosteroid isomerase [9]. In addition, several groups have used substrates based on α,β-unsaturated arylacryloyl esters to probe the active sites of serine and cysteine proteases [1,10-14]. Studies in our laboratory have shown that for a series of acylserine proteases a linear correlation exists between νc=o, the substrate’s carbonyl group frequency, and log k₃, where k₃ is the deacylation rate constant [2,15].

It is of significant interest to determine the energy of interaction between the substrate’s carbonyl group and an enzyme residue. If it is assumed that the substrate polarization results from hydrogen bonding with one or more enzyme donors, then the hydrogen bond enthalpy of this interaction can be determined by performing hydrogen bonding studies on simple model compounds. The model studies are performed using a compound that resembles the enzyme–substrate complex as closely as possible. In addition, it is of particular importance that the model compound is soluble in a non-polar solvent (CCl₄) so that the solvent does not compete for hydrogen bond formation between the model compound’s carbonyl group (acceptor) and the hydrogen bond donor. In this paper we describe hydrogen bonding studies on two esters, 5-methylthienylacryloyl methyl ester and thienylacryloyl methyl ester, shown in Scheme 1. These compounds are models of the acylserine proteases used to generate the linear correlation between νC=O and log k₃ described above [2,15].

2. Experimental methods

trans-5-Methylthienylacrylic acid was synthesized from malonic acid and 5-methyl-2-thiophene-carboxaldehyde (Aldrich) as described previously [16]. The imidazole derivative of the acid was generated using 1,1'-carbonyldiimidazole in dry THF. trans-5-Methylthienylacryloyl methyl ester (5MeTAOMe; λₘₐₓ 317 nm, acetonitrile) was synthesized by incubation of trans-5-methylthienylacryloylimidazole in methanol for 6 h followed by HPLC purification on a reverse phase column using a water–acetonitrile gradient [17]. Similarly, 1-13C=O trans-5-methylthienylacryloyl methyl ester (5MeTA-1-13C-OMe) was synthesized using 1,3-13C-malonic acid, and trans-5-methylthienylacryloyl-2-d methyl ester (CH=C=CD-C(=O)-; 5MeTA-2-d-OMe) was synthesized using malonic-d₃ acid-d₂ in the initial condensation with 5-methyl-2-thiophene-carboxaldehyde. Trans-Thienylacryloyl methyl ester (TAOMe; λₘₐₓ 306 nm, acetonitrile) was synthesized from 2-thiophene-carboxaldehyde as described above.

Products were analysed by exact mass analysis and NMR spectroscopy. NMR data were obtained in CD₃CN or CDCl₃. Data are reported as chemical shift in ppm, multiplicity, integration and coupling in Hz.

H-NMR: 5MeTAOMe (CDCl₃), δ 2.51 (d, 3, 1.0); 3.79 (s, 3); 6.11 (d, 1, 15.4); 6.71 (dd, 1, 1.0, 3.5); 7.06 (d, 1, 3.5); 7.71 (d, 1, 15.4). TAOMe (CD₃CN), δ 3.75 (s, 3); 6.28 (d, 1, 15.7); 7.11 (dd, 1, 3.6, 5.1); 7.40 (d, 1, 3.6); 7.54 (d, 1, 5.1); 7.81 (d, 1, 15.7).

For 5MeTA-2-d-OMe, 1H-NMR indicated that deuterium substitution at C₂ was greater than 85%.

The enthalpy and entropy of hydrogen bond formation were determined in CCl₄ in a manner...
similar to that described by Thijs and Zeegers-Huyskens [18]. Hydrogen bond donors were ethanol (pK 15.5), phenol (pK 9.99) and 3,5-dichlorophenol (pK 8.18), chosen to represent a large range of pK values in order to vary the hydrogen bond strength as much as possible.

To determine $-\Delta H$ and $\Delta S$, the concentration of donor was maintained at 6 mM for ethanol and 3 mM for phenol and 3,5-dichlorophenol, to avoid self-association. The concentration of acceptor was varied in the range 0–200 mM (TAOMe, ethanol), 0–89 mM (TAOMe, phenol), 0–51 mM (TAOMe, 3,5-dichlorophenol), 0–200 mM (5MeTAOMe, ethanol) and 0–30.3 mM (5MeTAOMe, 3,5-dichlorophenol). FTIR spectra were obtained using a Digilab FTS-60 spectrometer equipped with a DTGS detector. The sample cell had KBr windows and a path length of 0.5 mm. Temperature was controlled with a water bath using cryosolvent (30% ethylene glycol) circulating through the cell holder in the FTIR sample compartment. The temperature of the cell contents was measured using a thermocouple inserted into the cell through one of the cell’s Teflon plugs.

At the beginning of each temperature run, the cell was placed in the cell holder with the temperature bath set at 263 K. At thermal equilibrium, the temperature in the cell was 275 ± 1 K. After acquiring the first FTIR spectrum (64 scans), the bath temperature was changed to 273 K and a second FTIR spectrum was acquired after a 30 min waiting period. This process was repeated in 10 K increments up to a bath temperature of 323 K. Owing to the temperature gradient between the bath and the cell, the actual cell temperature measured at each point was (±1 K) 275, 280, 287, 295, 303, 310 and 318 K. For each donor–acceptor pair, five concentrations of acceptor were used over the concentration ranges listed above. After subtracting a solvent spectrum, recorded at the appropriate temperature, the concentration of free donor ($[\text{donor}]_{\text{free}}$) was determined by integrating $n_\text{O-H}$ for the free donor ($n_\text{O-H}$ free; ethanol 3632 and 3626 cm$^{-1}$; phenol 3609 cm$^{-1}$; 3,5-dichlorophenol 3599 cm$^{-1}$). Using $[\text{donor}]_{\text{free}}$, both $[\text{donor}]_{\text{bound}}$ and $[\text{acceptor}]_{\text{free}}$ were calculated and $K$, the equilibrium constant for hydrogen bond formation, was determined by plotting $[\text{donor}]_{\text{bound}}$ against $[\text{acceptor}]_{\text{free}} \times [\text{donor}]_{\text{free}}$. $K$ was determined at every temperature and $-\Delta H$ and $\Delta S$ were obtained using the Van’t Hoff equation, $\ln K = -\Delta H/RT + \Delta S/R$, by plotting in $K$ vs. $1/T$.

$\Delta n_\text{C=O}$, the decrease in carbonyl frequency upon hydrogen bond formation, was determined either by adding an excess of donor (0.1–0.2 M) such that $\approx 50\%$ of the acceptor was complexed or by interactively subtracting a FTIR spectrum of free acceptor from that of a solution of acceptor and donor in which only a small fraction of the acceptor was complexed. Using the latter interactive subtraction method, only a small amount of donor needed to be added, therefore ensuring that the carbonyl groups only had a single hydrogen bond donor.

Band fitting was performed with SPECTRACALC (Galactic Industries, NH).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The values of $\Delta n_\text{C=O}$, $-\Delta H$ and $\Delta S$ for each donor–acceptor hydrogen bonding pair</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta n_\text{C=O}/\text{cm}^{-1}$</td>
<td>$-\Delta H/(\text{kJ mol}^{-1})$</td>
</tr>
<tr>
<td>TAOMe + ethanol</td>
<td>19</td>
</tr>
<tr>
<td>5MeTAOMe + ethanol</td>
<td>21</td>
</tr>
<tr>
<td>TAOMe + phenol</td>
<td>25</td>
</tr>
<tr>
<td>TAOMe + 3,5-diClphenol</td>
<td>29, 26</td>
</tr>
<tr>
<td>5MeTAOMe + 3,5-diClphenol</td>
<td>33</td>
</tr>
<tr>
<td>5MeTA-2-d-OMe + methanol</td>
<td>18</td>
</tr>
<tr>
<td>5MeTA-2-d-OMe + ethanol</td>
<td>17</td>
</tr>
<tr>
<td>5MeTA-2-d-OMe + phenol</td>
<td>33</td>
</tr>
<tr>
<td>5MeTA-2-d-OMe + 3,5-diClphenol</td>
<td>33</td>
</tr>
</tbody>
</table>
3. Results and discussion

3.1. Determination of $-\Delta H$ and $\Delta S$

The values determined for $-\Delta H$ and $\Delta S$ for each donor–acceptor pair are given in Table 1. As expected, $-\Delta H$ increases as the acidity of the hydrogen bond donor increases. The two esters used, TAOMe and 5MeTAOMe, have similar hydrogen bonding acceptor propensities, as shown by the similarity of $-\Delta H$ determined for the two esters with ethanol ($-9.5$ and $-10.3$ kJ mol$^{-1}$, respectively) and 3,5-dichlorophenol ($-25.6$ and $-26.5$ kJ mol$^{-1}$, respectively).

3.2. Analysis of ester carbonyl profiles

In order to estimate $\Delta \nu_{C=O}$ it is important to understand the carbonyl profiles for both free and complexed esters. The carbonyl profile for 5MeTAOMe in CCl$_4$ and in the absence of donor is characterized by a major band at 1722 cm$^{-1}$ with a shoulder at 1707 cm$^{-1}$ (Fig. 1(a)). Similarly, the carbonyl profile for TAOMe is characterized by a band at 1724 cm$^{-1}$ with a shoulder at 1713 cm$^{-1}$ (Fig. 1(c)). The basis for interpreting the carbonyl profiles is in the quantum mechanical and vibrational spectroscopic work of Dulce G. Faria et al. on a number of $\alpha, \beta$-unsaturated esters, such as methyl cinnamate [19], methyl acrylate [20], and methyl trans-crotonate [21]. In the present context, the key finding of Dulce G. Faria et al. is that $\alpha, \beta$-unsaturated esters exist in two conformational populations about the C=C–C(=O)– single bond, namely the $s$-cis and $s$-trans shown in Scheme 2. The $s$-cis is the lower energy form and the carbonyl stretching frequency for that rotamer occurs at $\approx 10$ cm$^{-1}$ lower in frequency compared with the
s-trans form. With this information we assign the peak near 1720 cm⁻¹ in Figs. 1(a) and 1(c) to the s-trans rotamer of 5-MeTAOMe and the shoulder near 1707 cm⁻¹ to s-cis. In keeping with the s-cis being the more stable form, the shoulder increases in relative intensity as temperature is lowered (data not shown). The overall low intensity of the s-cis band is explained by hypothesizing that the carbonyl of this isomer undergoes a smaller change in dipole moment with the carbonyl stretching vibration and thus has lower intrinsic infrared absorbance compared with the carbonyl of s-trans.

Isotopic substitution was used to confirm the above interpretation of the carbonyl profile and to provide additional data for the hydrogen bonding studies. The analog 5MeTAOMe deuterated at the C₂ position (5MeTA-2-d-OMe) was analysed. For this ester νC=O is a broad, apparently single band at 1717 cm⁻¹ (Fig. 2). However, curve fitting of this band required two components to achieve a good fit, with peak maxima at 1719 and 1711 cm⁻¹ (Fig. 2). These values are close to those obtained for the unlabelled derivative, the band at 1719 cm⁻¹ being assigned to the s-trans conformer and the band at 1711 cm⁻¹ being assigned to the s-cis conformer.

Fig. 2. FTIR spectra of uncomplexed and hydrogen-bonded SMeTA-2-d-OMe in CCl₄. Spectra were obtained in a 0.05 mm path length cell fitted with KBr windows. The solvent spectrum has been subtracted from each spectrum. (a) 10 mM SMeTA-2-d-OMe; (b) 10 mM 5MeTA-2-d-OMe + 0.1 M ethanol. The spectrum of uncomplexed 5MeTA-2-d-OMe (spectrum (a)) has been subtracted using a scaling factor of 0.827; (c) 10 mM 5MeTA-2-d-OMe + 0.1 M phenol. The spectrum of uncomplexed 5MeTA-2-d-OMe (spectrum (a)) has been subtracted using a scaling factor of 0.467; (d) 10 mM 5MeTA-2-d-OMe + 0.1 M 3,5-dichlorophenol. The spectrum of uncomplexed 5MeTA-2-d-OMe (spectrum (a)) has been subtracted using a scaling factor of 0.182.
Table 2
ν<sub>νC=O</sub> values for "free" (non-hydrogen bonded) and hydrogen bonded carbonyls

<table>
<thead>
<tr>
<th></th>
<th>Free</th>
<th>Ethanol</th>
<th>Phenol</th>
<th>3,5-Dichlorophenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAOMe s-trans</td>
<td>1724</td>
<td>1705</td>
<td>1699</td>
<td>1695</td>
</tr>
<tr>
<td>s-cis</td>
<td>1713</td>
<td></td>
<td>1688</td>
<td>1687</td>
</tr>
<tr>
<td>5MeTAOMe s-trans</td>
<td>1722</td>
<td>1701</td>
<td>1690</td>
<td>1684</td>
</tr>
<tr>
<td>s-cis</td>
<td>1717</td>
<td>1704</td>
<td>1693</td>
<td>1685</td>
</tr>
<tr>
<td>5MeTA 2 d OMe</td>
<td>s-trans</td>
<td>1719</td>
<td>1696</td>
<td>1688</td>
</tr>
<tr>
<td>s-cis</td>
<td>1711</td>
<td></td>
<td>1677</td>
<td></td>
</tr>
</tbody>
</table>

3.3. Determination of Δν<sub>C=O</sub>

Δν<sub>C=O</sub> was determined by adding sufficient hydrogen bond donor to complex 50% of the ester (calculated from K). Data are shown in Fig. 1 for 5MeTAOMe with 3,5-dichlorophenol and for TAOme with phenol. Upon addition of 0.11 M 3,5-dichlorophenol to 20 mM 5MeTAOMe, a band is observed at 1689 cm<sup>-1</sup>. The interpretation is that the 1689 cm<sup>-1</sup> band represents the hydrogen bonded form of the species giving rise to the band at 1722 cm<sup>-1</sup> in the spectrum of the uncomplexed ester, giving Δν<sub>C=O</sub> = 33 cm<sup>-1</sup> in this instance. Curve fitting studies showed that the band at 1689 cm<sup>-1</sup> required only one component for adequate fitting. Similarly, a single band arising from the hydrogen bonding of 5MeTAOMe with ethanol was observed at 1701 cm<sup>-1</sup> (data not shown), giving Δν<sub>C=O</sub> = 21 cm<sup>-1</sup>.

The carbonyl profile for uncomplexed TAOme

Fig. 3. Plot of −ΔH vs. Δν<sub>C=O</sub> using the data presented in Table 1. The data have been fitted by linear regression to the equation −ΔH = 1.36Δν<sub>C=O</sub> − 16.10.
Table 3
Collated data relating $\Delta H$ and $\Delta \nu_{C=O}$: $-\Delta H$ (kJ mol$^{-1}$) = slope $\Delta \nu_{C=O}$ (cm$^{-1}$) + constant

<table>
<thead>
<tr>
<th>Acceptor and 5MeTAOMe$^a$</th>
<th>Slope</th>
<th>Constant</th>
<th>$r$</th>
<th>$-\Delta H$(kJ mol$^{-1}$) required to shift $\nu_{C=O}$ by 20 cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAOMe and 5MeTAOMe$^a$</td>
<td>1.36</td>
<td>-16.10</td>
<td>0.92</td>
<td>11.10</td>
</tr>
<tr>
<td>Methyl acrylate, methyl trans-crotonate, methyl trans-cinnamate$^b$</td>
<td>0.46</td>
<td>4.53</td>
<td></td>
<td>13.17</td>
</tr>
<tr>
<td>N,N-Dimethylacetamide$^c$</td>
<td>2.63</td>
<td>-42.63</td>
<td>0.833</td>
<td>9.97</td>
</tr>
<tr>
<td>Methyl acetate$^c$</td>
<td>1.67</td>
<td>-22.95</td>
<td>0.999</td>
<td>10.45</td>
</tr>
<tr>
<td>Acetophenone$^c$</td>
<td>1.28</td>
<td>-2.11</td>
<td>0.967</td>
<td>23.49</td>
</tr>
<tr>
<td>Benzophenone$^c$</td>
<td>1.49</td>
<td>-4.40</td>
<td>0.992</td>
<td>25.4</td>
</tr>
<tr>
<td>Acetone$^c$</td>
<td>1.96</td>
<td>-0.47</td>
<td>0.969</td>
<td>38.73</td>
</tr>
</tbody>
</table>

$^a$ This work.  
$^b$ Ref. [22].  
$^c$ Ref. [18].

is characterized by a band at 1724 cm$^{-1}$ with a shoulder at 1713 cm$^{-1}$ (Fig. 1(c)). Upon addition of 0.1 M phenol (Fig. 1(d)) a new band is observed at 1699 cm$^{-1}$. Curve fitting of this band required two components to achieve a good fit, with peak maxima at 1699 and 1688 cm$^{-1}$. The interpretation, consonant with the observations on methyl cinnamate [22], is that the bands at 1699 and 1688 cm$^{-1}$ represent the hydrogen bonded forms of the species giving rise to the carbonyl bands at 1724 and 1713 cm$^{-1}$, respectively, in uncomplexed TAOMe. This gives $\Delta \nu_{C=O}$ values of 25 and 25 cm$^{-1}$, respectively. Similarly, for TAOMe with 3,5-dichlorophenol, the hydrogen bonded carbonyl profile is composed of two components, at 1695 and 1687 cm$^{-1}$ (data not shown), giving $\Delta \nu_{C=O}$ of 29 and 26 cm$^{-1}$, respectively. When ethanol was used as hydrogen bond donor, only a single component was required to fit the hydrogen bonded carbonyl band, with a maximum at 1705 cm$^{-1}$. Assuming that the hydrogen bonded carbonyl arises from the uncomplexed species with $\nu_{C=O}$ 1724 cm$^{-1}$, this gives a $\Delta \nu_{C=O}$ of 20 cm$^{-1}$.

In order to confirm the $\Delta \nu_{C=O}$ values calculated for unlabelled 5MeTAOMe, $\Delta \nu_{C=O}$ was also determined for 5MeTA-2-d-OMe with ethanol, phenol and 3,5-dichlorophenol. The FTIR carbonyl profiles are shown in Fig. 2. For the spectra obtained in the presence of a hydrogen bond donor (Fig. 2(b,c,d)), the spectrum of uncomplexed 5MeTA-2-d-OMe has been subtracted, leaving $\nu_{C=O}$ arising from only the hydrogen bonded ester carbonyls. Curve fitting has been performed, each carbonyl band requiring two components for an adequate fit. For the deuterated analog, $\Delta \nu_{C=O}$ values calculated using peak maxima from the raw data are in good agreement with values calculated for the individual deconvoluted s-trans or s-cis features. The assignments for $\nu_{C=O}$ “free” and hydrogen bonded are given in Table 2. It can also be seen from Table 2 that there is good agreement between the $\Delta \nu_{C=O}$ values obtained with 5MeTA-2-d-OMe compared with those obtained with unlabelled 5MeTAOMe.

3.4. Relationship between $-\Delta H$ and $\Delta \nu_{C=O}$

Fig. 3 shows a plot of $-\Delta H$ against $\Delta \nu_{C=O}$. The plot is linear and fits to the equation $-\Delta H = 1.36\Delta \nu_{C=O} - 16.10$ ($r = 0.92$), where the units are kJ mol$^{-1}$ for $\Delta H$ and cm$^{-1}$ for $\Delta \nu_{C=O}$. These data have been used to quantitate hydrogen bonding strengths in the active sites of chymotrypsin and subtilisin [15].

Table 3 is a compilation of some of the quantitative data that exist relating $-\Delta H$ and $\Delta \nu_{C=O}$. It can be seen that significantly less energy is required to bring about a unit shift in the carbonyl frequency of $\alpha,\beta$-unsaturated esters compared with ketones (by a factor of 2-7), but that the values for saturated and unsaturated esters are similar. The data presented here are similar to the values reported for other $\alpha,\beta$-unsaturated esters by Dulce G. Faria et al. [22], demonstrating that the
substitution on the acryloyl moiety does not markedly change the inherent “stiffness” of the α,β-
unsaturated ester carbonyl.

Using the relationship we have established between $-\Delta H$ and $\Delta \nu_{C=O}$, we have quantitated
the change in $\nu_{C=O}$ observed through a series of acylserine proteases in which the acyl donor was
thienylacryloyl or 5-methylthienylacryloyl. This allowed us to estimate that the decrease in $\nu_{C=O}$
of 54 cm$^{-1}$ through the acyl enzyme series could be accounted for by an increase in hydrogen bond
enthalpy to the carbonyl oxygen of 57 kJ mol$^{-1}$ [2,15]. It should be noted that the values of $\Delta H$
given in Refs. [2] and [15] are low by a factor of 2.3, due to an arithmetic error.

References