Enthalpy and Volume Changes on the pH Jump Process Studied by the Transient Grating Technique

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A pH jump reaction of o-nitrobenzaldehyde is studied by using the time-resolved transient grating method. For low pH solutions, the grating signal due to the proton is observed clearly without any pH indicator. The origin of the signal is attributed to a pure volume grating, from which the partial molar volume of the proton can be determined accurately. In an alkali solution, the hydroxyl ion is observed as the main species of the grating signal. The enthalpy changes of the reaction in pH=6.5 and 8.2 are determined to be -131.2 and -133.6 kJ/mol, respectively, indicating that the reaction is exothermic. The negative volume change at the lower pH solutions is due to the electrostriction by the creation of the ionic species. At the higher pH, the reaction volume changes to positive, which is mainly due to the water formation. This pH jump reaction was used to study the unfolding process of apomyoglobin.

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A pH jump reaction, which can suddenly change the proton concentration in solution, is important not only as prototype chemical reactions, but also they have many unique applications such as to studies of the kinetics of protein folding in solution. Considering a rather slow folding process, we should develop a pH jump technique using an irreversible reaction. A photodissociation reaction of o-nitrobenzaldehyde (o-NBA) is one of such reactions. Upon the photoexcitation of o-NBA, the aci-form is rapidly produced through the intramolecular proton transfer reaction and the proton is dissociated to induce the nitronate anion in methanol and water (Scheme 1). Consequently the nitronate anion is converted to the o-nitrosobenzoic anion (NS-) with a quantum yield of about 0.4 in methanol and water.1

In this paper, we study the photodissociation reaction of o-NBA by the time-resolved transient grating (TG) technique; one of powerful spectroscopic methods to trace the dynamics of the energy and the volume change. The merit of the TG technique to study such a proton releasing reaction will be discussed.

Experimental

The experimental setup for the TG and photoacoustic (PA) experiments was similar to those reported previously.2,3 The third harmonic of a Nd-YAG laser with a 10 ns pulse was used as an excitation beam and a He-Ne laser beam (633 nm) as a probe beam. Photoacoustic signals generated by the photexcitation were detected by a piezoelectric transducer (PZT). The sample solution was changed to a fresh one after every 200 shots of the excitation laser pulses. The repetition rate of the excitation laser was about 1 Hz and the sample was gently stirred during measurement to prevent possible bleaching of the reactant and to dissipate photoproduct away from the excitation region. The absorbance of the samples in the TG and PA experiments were kept at about 0.5 at 355 nm, the excitation wavelength.

Results and Discussion

Photodissociation of o-NBA

The TG signals after the photoexcitation of o-NBA in various pH solutions under the nitrogen-saturated condition are shown in Fig.1. The initial strong spikelike signal comes from the thermal grating, which is produced by the thermal energy coming from the nonradiative transition and the enthalpy change of the reaction. The features of the signal after the thermal grating depend on the pH of the solution. The thermal grating signal decays to the baseline and a grow-decay curve is observed for the low pH solution (pH≤8.2), while for pH=10.4 solution, the thermal grating signal does not decay to the baseline but continuously decays slowly to the baseline and then show a grow-decay curve. In the following, we interpret these changes of the grating signal by considering the chemical species involving the reaction.

The TG signal intensity is proportional to the sum of the squares of the refractive index change and the absorbance change, which are induced by the thermal energy (thermal grating) and by chemical species created (or depleted) after the photodissociation reaction of o-NBA (species grating). Since absorption of any chemical species in this reaction is negligible at the probe wavelength (633 nm), the refractive index change is the main contribution to the signal. The temporal profile of

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Figure 1. Time profile of the TG signals after photoexcitation of o-NBA in various pH solutions

The whole time range can be reproduced by a sum of three exponential functions in any solutions. All of the decay rate constants depend on $q^2$ (q: grating wave vector), indicating that the decay of the signal is governed by the diffusion process in the solution. Therefore, the TG signal can be expressed by

\[
I_{TG}^{1/2} = \alpha \left( \delta n_{nth}^o \exp(-D_{nth}q^2t) + \delta n_{nf}^o \exp(-D_fq^2t) + \delta n_{ns}^o \exp(-D_sq^2t) \right)
\]

where $\alpha$ is a constant, $\delta n_{nth}^o$, $\delta n_{nf}^o$, and $\delta n_{ns}^o$ are initial refractive index changes of the thermal grating and the species gratings of the fast and slow decay components, respectively. $D_{nth}$, $D_f$, and $D_s$ are the thermal diffusivity of the solution, the diffusion constants of the fast and slow decay components, respectively.

We identify the chemical species by the sign of $\delta n$ and the diffusion constant. From the slope of the plot of the decay rates of the fast and slow developing species grating signal against $q^2$, $D_{nth}$ and $D_f$ in pH=4.1 solutions are determined to be 11.8 and 0.45 x 10^{-9} m^2/s, respectively. Considering the reaction scheme (Scheme 1), we expect that the proton (H$^+$), NS$^-$ and o-nitrosobenzoic acid (NS) can contribute to the species grating signal. The diffusion constant of the fast component is remarkably larger than that expected for NS$^-$ or NS but rather close to the reported of proton (D_H$^+$) in water (9.0-10 x 10^{-9} m^2/s at 25°C). Considering the magnitude of D, we can assign the chemical species of the fast decay component to H$^+$ definitely and the slow one to NS$^-$. The spatial modulation of the hydroxyl ion is created by the water formation reaction with the proton from o-NBA

\[
H^+ + OH^- \rightleftharpoons H_2O
\]

Because of this reaction, the concentration of OH$^-$ in the light illuminated region in the grating pattern decreases. Therefore the phase of the spatial modulation of the hydroxyl ion should be 180° shifted from the optical grating.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>D_H$^+$</th>
<th>D_NS$^-$</th>
<th>D_OH$^-$</th>
<th>D_o-NBA</th>
<th>D_Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH=4.1</td>
<td>11.8</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH=6.5</td>
<td>8.7</td>
<td>0.68</td>
<td></td>
<td></td>
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<tr>
<td>pH=8.2</td>
<td>1.9</td>
<td>0.77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH=10.4</td>
<td>2.4</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Enthalpy and Volume Changes

The magnitude of $\delta n_{\text{th}}$ is given by

$$\delta n_{\text{th}} = \frac{dn}{dT} \frac{hv\phi W}{\rho C_p} \Delta N$$

(2)

where $dn/dT$ is the temperature dependence of the refractive index, $hv$ is the photon energy of the excitation energy, $\phi$ is the quantum yield and $W$ is the molecular weight. The enthalpy change of this photodissociation reaction can be determined by the quantitative measurement of the thermal grating intensity. The thermal grating intensity of $\alpha$-NBA in pH=6.5 and 8.2 is compared with that of a reference sample, bromocresol purple, which gives rise to only the thermal grating signal with a unit quantum yield of the nonradiative transition within the pulse width of the excitation laser. From the thermal grating intensity, $\phi \Delta H$ in pH=6.5 and 8.2 solutions are determined as $-52.5$ and $-53.5 \pm 0.5 \text{ kJmol}^{-1}$, respectively. Using the reported quantum yield, $\phi = 0.4$, $\Delta H$ in pH=6.5 and 8.2 are estimated to be -131 and -134 kJmol$^{-1}$, respectively.

The measured $\Delta H$ in pH=6.5 is significantly larger than that reported by Carcelli et al. They reported $\Delta H$ for the photolysis of $\alpha$-NBA in neutral aqueous solutions ([NaCl]=100mM) as $-88.45 \text{ kJmol}^{-1}$ by the temperature dependence of the photoacoustic (PA) signal intensity in a range of 4-15 °C. In the PA method, $\Delta H$ and $\Delta V$ are always assumed to the temperature independent. If they are not, the signals from the thermal contribution and volume contribution are significantly mixed and the values are not accurate. In spite of this assumption, they actually reported anomalous temperature dependence of $\Delta H$. This fact indicates that the previous analysis may not be correct. Therefore, the difference between $\Delta H$ from the TG and from the PA methods may be due to this temperature dependent $\Delta H$.

From the acoustic signal intensities of the sample and the reference sample, and with $\Delta H$ from the TG measurements, we obtain the volume change ($\Delta V$) in pH=6.5 and 8.2 to be $-5.3$ and $-7.5 \text{ ml/mol}$, respectively. The $\Delta V$ corresponds to the molecular volume change and the solvation of the photogenerated ions. These values are consistent with the previously reported values of $-5.2 \text{ ml/mol}$ at pH=6.7 and $-7.1 \text{ ml/mol}$ in neutral aqueous solutions ([NaCl]=100 mM).

On the other hand, using the mean value of $\Delta H$ measured in pH=6.5 & 8.2, the $\Delta V$ of 15.6 ml/mol in pH=10.4 is estimated, indicating that the neutralization reaction of the photodissociated proton with hydroxyl ion leading to the water formation takes place substantially. Indeed, this $\Delta V$ is close to the sum of the volume change obtained at pH=6.5 and the reported volume change of the water formation $H^+ + OH^- \rightarrow H_2O, \Delta V = 22.4 \text{ cm}^3/\text{mol}$.

Finally, the photodissociation reaction of $\alpha$-NBA was used to study the unfolding process of horse-heart apomyoglobin (ApoMb). Compared to the TG signal of $\alpha$-NBA, a very strong TG signal, which consists of the thermal grating and the species gratings of the rise and decay components, is observed in aqueous solution of $\alpha$-NBA and ApoMb in a longer time scale (Fig. 2). From the slopes of the plot of the decay rate constant vs $\log \text{[Urea]}$, the diffusion constants of the rise and decay components are determined to be 1.4 and $0.18 \times 10^{-9} \text{ m}^2/\text{s}$, respectively.

From the magnitude of $D$, we can assign the rise and decay components to $\alpha$-NBA and ApoMb, respectively. The strong TG may be attributed to a conformational change of ApoMb from the native to unfolding state (or intermediate) induced by the pH jump process or to an intermolecular reaction between the $\alpha$-NBA product and ApoMb. The exact origin of the TG signal from this ApoMb system will be published in the future. Interestingly, D of the slow decay component is significantly decreased by the addition of urea, which is well-known as a denaturant. This change supports that the origin of the slow decay component is attributed to the diffusion of ApoMb (See Fig. 2).

![Figure 2](image-url)