Photochemical Reaction of 2-Nitrobenzaldehyde by Monitoring the Diffusion Coefficient

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The photochemical reaction of 2-nitrobenzaldehyde (NBA) in various electrolyte solutions as well as in a solution with a protein is investigated from the viewpoint of the diffusion coefficient by using the time-resolved transient grating (TG) method. For studying reaction with optically silent materials such as electrolytes, we found that the diffusion coefficient is useful for identifying chemical species involved in the reaction. The TG signal that represents the diffusion process was dramatically changed by adding electrolytes such as Cl^- or Br^-, and this result indicates that these electrolytes should participate in the photochemical reaction of NBA. It was found that the time profile of the grating signal also depends on the addition of guanidine−HCl or apomyoglobin. The structural change of the protein associated with the NBA reaction is discussed.

1. Introduction

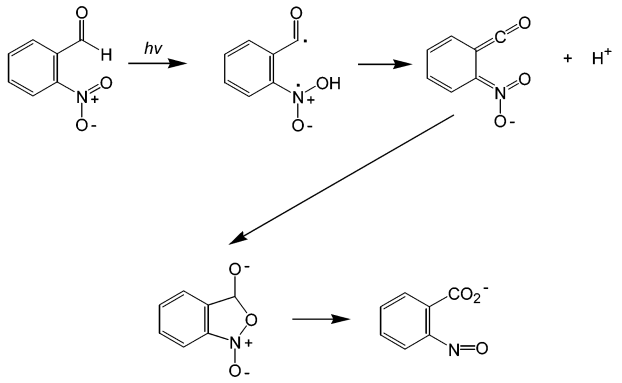
Since caged compounds can control the concentration of a bioactive substance such as proton,[1−3] ATP,[4] GTP,[5] and Ca^{2+},[6] etc., spatially as well as temporally by photoirradiation, a variety of caged compounds have been developed and widely utilized in biophysical, biochemical, physiological, or cell biological systems. Understanding the substance-releasing process is essential to correctly interpret observations in these studies. In many cases, caged compounds have a 2-nitrobenzyl group, which is a major photolabile precursor. For example, a photodissociation reaction of 2-nitrobenzaldehyde (NBA) can change the proton concentration.[1−3] Since this is the simplest and fundamental caged compound, the reaction of NBA should be clarified in order to elucidate the mechanism of the substance releasing of the other caged compounds. In particular, the caged reactions are frequently used with the presence of ions or proteins for biological research, and, therefore, the study of the reaction under these conditions is unavoidable.

For studying chemical reactions, the transient absorption method has been frequently used and it is certainly a very powerful and informative technique. The fundamental reaction scheme of NBA has been reported by the transient absorption method.[1] Upon the photoexcitation of NBA, the aci-form is rapidly produced through the intramolecular proton-transfer reaction and a proton is released to yield the nitronate anion in aqueous solution (pH jump). Consequently the nitronate anion is converted to 2-nitrosobenzoic anion (NS^-) with a quantum yield of about 0.4 (Scheme 1).

When this reaction takes place in a buffer solution for a biological application, many ions or proteins should be contained in the solution and the reaction mechanism could be different from that in aqueous solution. The contribution of these ions to the reaction scheme should be clarified. However, it is rather difficult to detect the dynamics of such ions by the transient absorption method because these species absorb light in the far-UV region, and the absorption measurement in this region is frequently disturbed by the presence of many absorption bands from many species including NBA itself. Therefore it is desirable if there is another independent method that can detect such optically silent materials. In this respect, the detection of molecular volume change or diffusion coefficient (D) could provide a very unique method to clarify the reaction involving such ions. One prominent example recently shown is a study of the kinetics of a proton released from NBA.[2,7] There is no light absorption band of proton, and the presence is very difficult to detect without adding a pH sensitive dye, which could change the kinetics of the proton. However, even in such a case, we found that the presence of the proton can be identified by the diffusion coefficient and that the time development of the proton concentration can be monitored by the volume contribution without any pH indicator reagent. In this paper, we investigate the reaction of NBA in various solutions with the presence of ions and a protein, apomyoglobin (Mb), by monitoring D. The diffusion coefficient was sensitively detected in a time domain by the transient grating (TG) technique. We found evidence from the D measurement that the electrolyte ions are involved in the NBA reaction.

Previously the NBA photoreaction was used to study the protein folding process induced by the pH jump in the presence of guanidine hydrochloride (GdnHCl).[3,8] Protein folding of

SCHEME 1. Photoreaction of NBA



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apomyoglobin (Mb)[3] and cytochrome *c* (Cyt *c*)[8] were studied by the photoactoustic (PA) method and transient absorption method, respectively. However, in their analyses, the photochemical reaction of NBA is assumed to be the same as that in aqueous solution. Our study here suggests that the NBA photochemical reaction depends on the presence of electrolytes and that the NBA reaction with proteins should be reexamined.

2. Experimental Section

The experimental setup for the TG and PA experiments were similar to those reported previously.[9,10] The third harmonic of a Nd:YAG laser (Spectra-Physics Quantum-ray Model GCR-170-10) with a 10 ns pulse was used as an excitation beam and a He−Ne laser beam (633 nm) as a probe beam. The diffracted probe beam was isolated from the excitation laser beam with a glass filter (Toshiba R-60) and a pinhole, detected by a photomultiplier tube (Hamamatzu R-928), and the signal was fed into a digital oscilloscope (Tetronix TDS-520). The TG signal was averaged by a microcomputer to improve a signal-to-noise ratio (*S*/*N*). Photoacoustic signals were detected by a piezoelectric transducer (PZT). The signal was directly detected by the digital oscilloscope. The energy entering the sample was adjusted below 5 μJ/pulse. 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 3 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 size of the excitation beam at the sample position was ca. 1 mm pinhole diameter. The irradiated volume is small (typically ca. 4×10^{-3} cm^3) compared with the entire volume of the sample solution. All measurements were carried out at room temperature.

2-Nitrobenzaldehyde and guanidine hydrochloride were obtained from Nacalai Tesque Inc. Horse-heart apomyoglobin was obtained from Sigma Co. NBA was recrystallized from distilled water before use. The sample solution was prepared in a dark room just before the measurement. The absorbance of NBA used in the transient grating and photoacoustic experiments was kept at about 0.4 at 355 nm, the excitation wavelength.

The value of the grating wavnumber (q) was determined from the decay rate of the thermal grating signal of a calorimeter reference sample, bromocresol purple in aqueous solution, which gives rise to only the thermal grating signal due to the nonradiative transition within the pulse width of the excitation laser.

3. Method

Spatially modulated light intensity is created in the sample solution by crossing two coherent light beams. The TG intensity under the experimental condition is proportional to the sum of squares of the refractive index and the absorbance changes, which are induced by the spatially modulated light. Since absorptions of any chemical species in this reaction are negligible at the probe wavelength (633 nm), the refractive index change is the main contribution to the signal. The refractive index change mainly comes from the thermal energy releasing (thermal grating) and created (or depleted) chemical species by the photodissociation reaction of NBA (species grating). In the species grating signal, there are mainly two contributions. One is the refractive index change (δn) due to the volume change (volume grating), and the other is δn associated with the absorption change (population grating). Since the rate constant of the thermal grating signal should be $D_{th}q^2$ (D_{th}, thermal diffusivity of the solution; q, grating wavenumber), it is easy to distinguish the thermal grating component from the species grating signal. The TG signal can be expressed as follows.

$$I_{TG} = \alpha[\delta n_{th} \exp(-D_{th}q^2 t) + \sum_i \delta n_i \exp(-k_i t)]^2 \quad (1)$$

where α is a constant, δn_{th} and δn_i are the refractive index changes of the thermal grating and the species gratings of i species, respectively, and k_i is the rate constant of the component. When the main absorption bands are located on the blue side of the probe light (as in this case), δn_i of a reaction product is positive in many cases. On the other hand, δn_i of a reactant becomes negative because the phase of the spatial concentration modulation is shifted 180° from that of the light intensity.

The time evolution of the species grating signal intensity depends on the diffusion process of the species and concentration change due to a chemical reaction. If the concentration of the i-species disappears with a rate constant of k_d, the decay rate constant of the grating signal is given by

$$k_i = D_i q^2 + k_d \quad (2)$$

where D_i is the diffusion constant of the chemical species. Thus, the diffusion constant of each component can be determined from the slope of the plot of k_i vs q^2.

The magnitude of δn_{th} is given by

$$\delta n_{th} = \frac{dn}{dT}\frac{h\nu\phi W}{\rho C_p}\Delta N \quad \left(\phi = \frac{h\nu - \Phi\Delta H}{h\nu}\right) \quad (3)$$

where dn/dT is the temperature dependence of the refractive index, $h\nu$ is the photon energy of the excitation energy (337 kJ/mol), ΔN is a number of the reactive molecules in the unit volume, ρ is the density, C_p is the heat capacity at a constant pressure, Φ is the quantum yield of the reaction, and W is the molecular weight. ΔH is the enthalpy change from the reactant to the product. Thus, the enthalpy change of this photodissociation reaction can be determined by the quantitative measurement of the thermal grating intensity.

The molecular volume change, $\Phi\Delta V$, is determined from the PA intensity and ΔH from the TG experiment. The PA intensity (I_{PA}) is given by

$$I_{PA} = A'\Delta N|(h\nu + \Phi\Delta H)\left(\frac{W\beta}{\rho C_p}\right) + \Phi\Delta V| \quad (4)$$

where A' is a proportional constant which includes the sensitivity of the apparatus and β is the thermal expansion coefficient.

4. Results and Discussion

4.1. Photochemical Reaction of NBA. The TG signal of NBA in aqueous solution has been presented previously.[2] First we describe essential features of the TG signal after the photoexcitation of NBA in aqueous solution (pH 6.5) for a comparison purpose in different solutions (Figure 1 in ref 2). The signal consists of the thermal grating and species grating signals. The time profile of the signal in the whole time range can be fitted by a sum of three exponential functions. Since the rate constant of the fastest decay on a microsecond time scale agrees well with $D_{th}q^2$, it was attributed to the thermal grating signal due to the thermal energy by the nonradiative transition. The following signal in the slower time region is due to the species grating signal, which represents the volume change and absorption change. All of the rate constants are proportional to

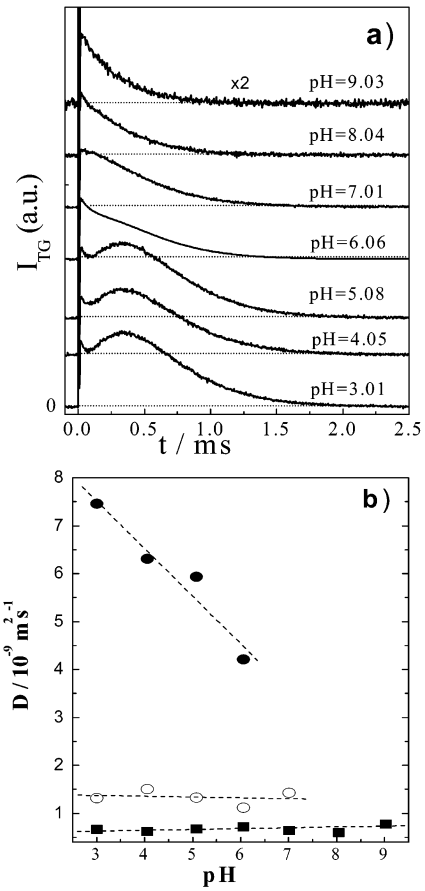

Figure 1. (a) pH dependence on the time profiles of the TG signals after photoexcitation of NBA in Tris-HCl buffer solutions and (b) changes of the diffusion constant of the rise component (○) and the fast (●) and the slow (■) decay components measured by the TG method as a function of the pH of the solution.

q^2. Therefore, the time profile of the TG signal can be expressed by

$$I_{TG} = \alpha[\delta n_{th}^{\circ} \exp(-D_{th}q^2t) + \delta n_f^{\circ} \exp(-D_fq^2t) + \delta n_s^{\circ} \exp(-D_{ts}q^2t)]^2 \quad (5)$$

where D_f and D_s are the diffusion constants of the fast and slow decay components, respectively. From the fitting of the signal, we determined D_f and D_s to be 8.7 and 0.68 $\times$ 10^{-9} m^2 s^{-1}, respectively. The very large D_f value indicates that this diffusing species should be unambiguously attributed to the proton released by this reaction. The diffusing species with the smaller D_s should be attributed to the product of NBA, nitrosobenzoic anion (NS^-). It should be noted that the presence of the proton is clearly observed by the volume contribution without any pH indicator. (See ref 2 for details.)

The TG signals of NBA in 100 mM Tris-HCl buffer solution are very much different from that in the aqueous solution, and the signals as a function of pH are shown in Figure 1a. The pH of the buffer solutions was adjusted with concentrated HCl solution. The initial strong spikelike signal at $t \sim 0$ is the thermal grating signal. In a region lower than pH 6, the signal in the whole time range can be fitted by a sum of four exponential functions. All of the decay rate constants (k) are proportional to q^2, indicating that the decay of the signal is governed by the diffusion process in the solution. Therefore, the TG signal can be expressed as follows.

$$I_{TG} = \alpha[\delta n_{th}^{\circ} \exp(-D_{th}q^2t) + \delta n_f^{\circ} \exp(-D_fq^2t) + \delta n_I^{\circ} \exp(-D_Iq^2t) + \delta n_s^{\circ} \exp(-D_sq^2t)]^2 \quad (6)$$

where δn_{th}°, δn_f°, δn_I°, and δn_s° are initial refractive index changes of the thermal grating and the species gratings of the fast, intermediate, and slow components, respectively. D_f, D_I, and D_s are the diffusion coefficients of the fast, intermediate, and slow components, respectively.

As shown in Figure 1a, the TG signal is dramatically changed with the change of the pH of the solutions. Namely, while all components (the fast, intermediate, and slow components) are observed in low-pH solutions ($\leq$pH 6), only the slow component is observed in alkali solutions. This pH dependence of the TG signal should be interpreted by considering the chemical species involved in this reaction. (The reason for the weaker signal intensity at a high pH is not known at present. It could be due to a low reaction quantum yield in the solution.)

The diffusion coefficient of each component was determined from the slope of the plot of the decay rate constants vs q^2, and they are shown in Figure 1b as a function of pH. It is worth noting that D of the fast component is significantly decreased by increasing the pH (from 7.5 $\times$ 10^{-9} m^2 s^{-1} (pH 3) to 4.2 $\times$ 10^{-9} m^2 s^{-1} (pH 6)), while the other, intermediate and slow, components are little affected (D_I = (1.30 $\pm$ 0.14) $\times$ 10^{-9} m^2 s^{-1} and D_s = (0.69 $\pm$ 0.07) $\times$ 10^{-9} m^2 s^{-1}). Considering the sign of δn and the diffusion coefficient, we can assign the chemical species of the fast and slow decay components to the proton (H^+) and the anion photoproduct (NS^-), respectively. The decrease of D of the proton with increasing pH may result from the electrostatic interaction between the proton and the anion species such as NS^- and Tris-HCl buffer, which can act as a proton acceptor. This pH dependence was also observed in aqueous solutions previously.[2]

It is interesting to note that the intermediate component appears in a range of pH $\leq$ 6. We have to consider the molecular origin of this component. The first clue for the assignment comes from the sign of the refractive index change of this component. Since the refractive index change of the thermal grating is negative at this temperature ($\delta n_{th}^{\circ} < 0$) and the interference dip between the thermal grating signal and species grating signal was observed in all solutions, the fast and slow components should possess a positive sign of the refractive index change (δn_f° and $\delta n_s^{\circ} > 0$), while the intermediate component has a negative sign ($\delta n_I^{\circ} < 0$). Here, it is worth noting that the positive and negative δn represent the creation and the depletion of the molecules, respectively. Therefore, the negative sign of this component ($\delta n_I^{\circ} < 0$) implies that this diffusing chemical species is depleted by this reaction.

The chemical species should be identified by the experimentally determined D. D of some chemical species contained in this solution and also the other solutions we investigated in this study are listed in Table 1 from selected literature.[11] Since we could not find the D of Tris ($C(NH_3)(CH_2OH)_3$) in the literature, we assume that the D of Tris is close to the D of triethanolamine ($N(CH_2CH_2OH)_3$). The chemical species contained in this solution are H^+, NBA, NS^-, Tris, and Cl^-. D of the intermediate component is certainly larger than those of triethanolamine, NBA, and NS^- and significantly smaller than that of the proton. Hence, this diffusing species cannot be these molecules or proton. D_I is also somewhat smaller than the D of Cl^- in aqueous solutions at infinite dilution. However, it is widely recognized that the ion diffusion is retarded by the relaxation effect and electrophoretic effect in the presence of the counterion. Therefore, it may be reasonable to assign the chemical species of the

TABLE 1: Diffusion Coefficients ($D/10^{-9}$ m^2 s^{-1}) of Selected Chemical Species Used in This Study in Aqueous Solution at Infinite Dilution[a]

	D		D
Cl^-	1.95	Na^+	1.33
Br	1.8	NH_4^+	1.96
K^+	1.96	$N(CH_2CH_2OH)_3$	0.48
Ca^{2+}	0.76		

[a] Reference 11.

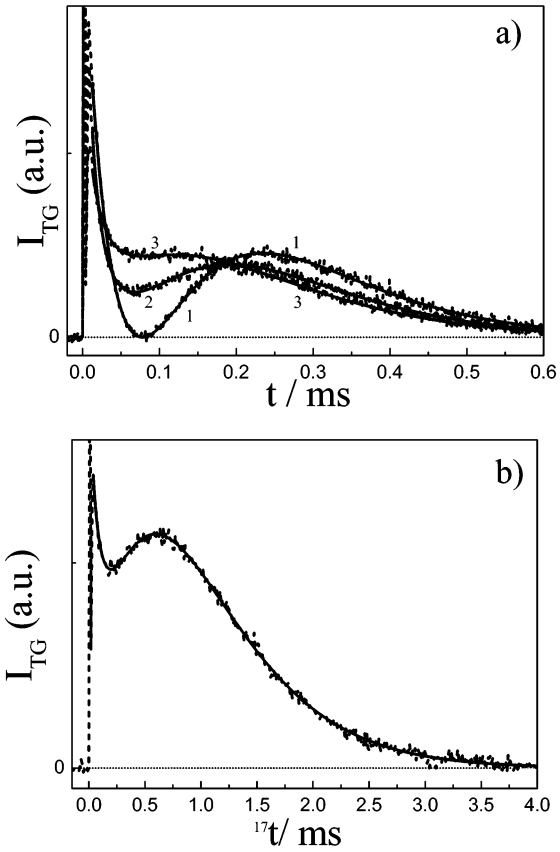

Figure 2. (a) TG signals (dotted lines) of NBA with some electrolytes in the solutions (1), NBA + NaCl (2), and NBA + NaBr (3) aqueous solutions ($q^2 = 5.63 \times 10^{-12}$ m^{-2}), and (b) TG signals of NBA in aqueous solutions with $CaCl_2$ ($q^2 = 1.24 \times 10^{-12}$ m^{-2}). The concentration of these electrolytes is adjusted to be 100 mM. The best fitted signals with three-exponential functions are shown by the solid line.

intermediate component to chloride ion. This assignment will be further tested in the next section. The observation of the intermediate component in the TG signal indicates that chloride ion participates in this reaction. This is a very unique advantage of the diffusion detection method because chloride ion is very difficult to detect by the optical absorption technique.

The negative sign of the intermediate component and the appearance of the decay components in the species grating signal imply that a free chlorine ion is depleted by the reaction, and it suggests that chloride ion is combined with the reaction product of NBA. Although the oxygen atom of the intermediate during the photoreaction of NBA has a negative charge, a positive charge on the nitrogen atom (Scheme 1) can be a source of the attractive interaction with an anion such as Cl^-. We suggest that the chloride ion diffuses with the intermediate species or the final product of NBA.

4.2. Electrolyte Effect on NBA Reaction. The ionic strength caused by the presence of electrolytes in solutions can affect a protein−protein interaction[12] and a conformation of a protein.[13−15] Therefore, the ionic strength is frequently varied in many biological studies. In the previous section, we found that the electrolyte, Cl^-, was involved in the NBA reaction. It is very important to understand an effect of other electrolytes on the photoreaction process of NBA.

TABLE 2: Diffusion Coefficients ($D/10^{-9}$ m^2 s^{-1}) Determined from the TG Signal of NBA in Various Solutions (100 mM)

	D_f	D_I	D_s
NH_4Cl	8.1 ± 0.2	1.4 ± 0.2	0.70 ± 0.1
NaCl	7.7 ± 0.2	1.2 ± 0.1	0.80 ± 0.1
NaBr	6.5 ± 0.2	1.2 ± 0.1	0.85 ± 0.1
KCl	7.7 ± 0.2	1.2 ± 0.1	0.80 ± 0.1
$CaCl_2$	8.3 ± 0.2	1.3 ± 0.2	0.75 ± 0.1

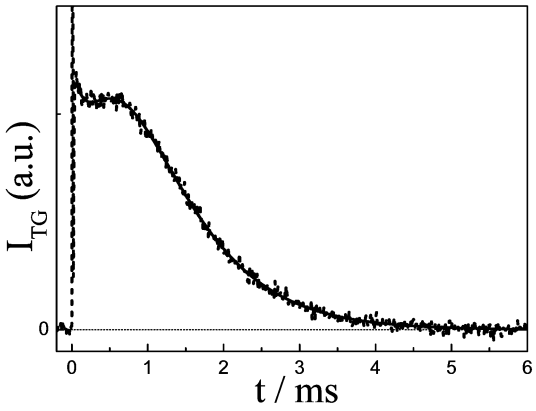

Figure 3. Time profiles of the TG signal (dotted lines) after the photoexcitation of NBA with GdnHCl (0.2 M). The best fitted signals with three-exponential functions are shown by the solid line.

We measured the TG signal of NBA in the presence of some electrolytes such as NaCl, NaBr, KCl, NH_4Cl, and $CaCl_2$. When these electrolytes were added in NBA aqueous solutions, the TG signals were sensitively changed, as shown in Figure 2. All of the decay rate constants depend on q^2, indicating that the decay of the signal is governed by the diffusion process in the solution. The TG signals of NBA in the presence of these electrolytes are very similar to those observed in Tris-HCl buffer solutions (≤pH 6). Therefore, all TG signals were also expressed by eq 5. From the rate constants at various q^2, D_f, D_I, and D_s are determined and listed in Table 2. From the magnitude of D and the sign of δn ($\delta n > 0$), we can easily assign the chemical species of D_f and D_s to the photoreleased proton and NS^-.

We should note that the intermediate component appears in all cases. The determined D_I is very similar in all solutions. Considering that D of Br^- is close to Cl^- (1.8×10^{-9} m^2 s^{-1}),[11] the intermediate component is consistently explained by the contribution of the anions in the solution, as suggested in the previous section.

We also examined the TG signal of NBA reaction with 0.2 M GdnHCl (Figure 3) that is frequently used for studying a protein folding reaction. The TG signal of NBA measured in the presence of GdnHCl is very similar to those observed in Tris-HCl buffer solution and by the presence of some electrolytes. Furthermore, the whole signal can be also fitted by a sum of four exponential functions, as shown in eq 6. From the slopes of the plot of the rate constant vs q^2, D_f, D_I, and D_s are determined to be $(7.7 \pm 0.2) \times 10^{-9}$, $(1.6 \pm 0.2) \times 10^{-9}$, and $(0.62 \pm 0.2) \times 10^{-9}$ m^2 s^{-1}, respectively. From the magnitude of D and the sign of δn ($\delta n < 0$), we can assign the chemical species of D_f, D_I, and D_s to the photoreleased proton, Cl^-, and NS^-, respectively.

4.3. NBA Reaction with a Protein. Not only are the photochemical reactions of NBA important as prototype pH

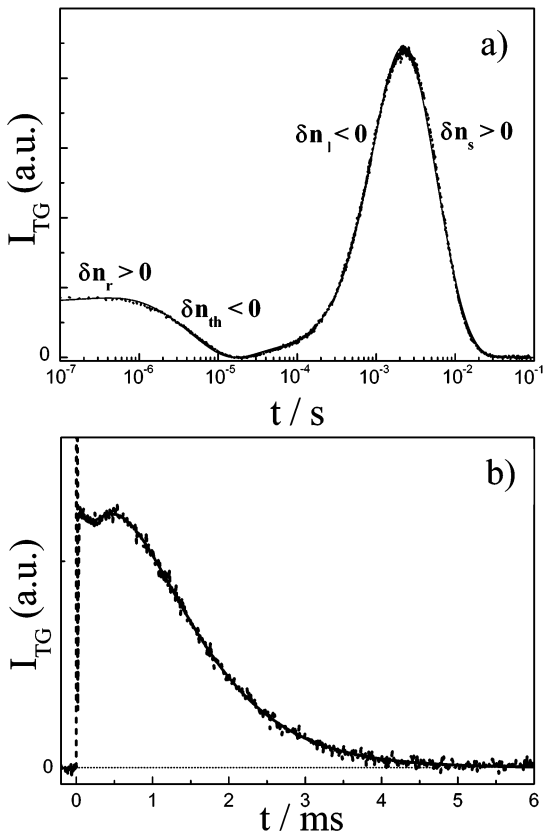

Figure 4. Time profiles of the TG signal (dotted lines) after the photoexcitation of NBA with Mb in aqueous solution (a) and 0.2 M GdnHCl solution (b). The best fitted signals with three-exponential functions are shown by the solid lines.

jump chemical reactions, but also they have many unique applications such as to investigations of the dynamics of pH-dependent phenomena. One of interesting applications may be a study of protein folding kinetics. This proton releasing reaction was applied to the pH change of a solution containing Mb and Cyt *c* to monitor the protein unfolding dynamics of Mb and Cyt *c* by the PA method and transient absorption method, respectively.[3,8] To study the NBA reaction with the presence of a protein, we investigated the TG signal with Mb in NBA aqueous solution.

We found that the TG signal changed dramatically by adding the protein as shown in Figure 4. The thermal grating signal rises with a rate determined by the pulse width and then shows a very weak slow rise. After the thermal grating signal decays to the baseline, the species grating signal shows another grow−decay feature. The rate constants of all components except the slow-rise component of the thermal grating are proportional to q^2, indicating that the slow-rise component observed in the thermal grating reflects a kinetic of reaction with the protein and that the decay of the signal is governed by the diffusion process in the solution. Therefore, the species grating signal after the complete decay of the thermal grating signal can be expressed as follows.

$$I_{TG} = \alpha[\delta n_I^\circ \exp(-D_I q^2 t) + \delta n_s^\circ \exp(-D_s q^2 t)] \quad (7)$$

From the slopes of the plot of the decay rate constant vs q^2, D_I and D_s are determined to be $(1.39 \pm 0.15) \times 10^{-9}$ and $(0.14 \pm 0.02) \times 10^{-9}$ m^2 s^{-1}, respectively. The determined D_s is much smaller than that expected for NBA (0.7×10^{-9} m^2 s^{-1}) and NBA related molecules but rather close to that of Mb (D_{Mb}) reported previously ($(0.11-0.12) \times 10^{-9}$ m^2 s^{-1}).[16−19] Therefore, we attribute this component to the species grating signal due to Mb. The species of D_I is again attributed to Cl^-, which should be contained in the Mb sample.

Recently, Abbruzzetti et al. studied the protein unfolding reaction of Mb using the laser-induced pH jump reaction of NBA. They used 0.2 mM GdnHCl solution to partially destabilize the protein and shift the pK_a for the native to intermediate transition neutrality. The observed photoacoustic signal after the photoexcitation of NBA + Mb in the presence of GdnHCl was analyzed with three-exponential kinetics. The thermal energy and volume changes associated with these steps were determined by the temperature variation method of the signal. The slowest change was explained by the volume and enthalpy changes due to the protein conformational change induced by the protonation reaction of Mb after the pH jump reaction. For comparison purpose, we measured the TG signal under the same condition ([Mb] = 40 μM and [GdnHCl] = 0.2 M). Surprisingly, we found that the species grating due to Mb disappeared when GdnHCl was added in the solution (Figure 4b).

From the amplitude of the thermal grating signal, we determined the enthalpy change ($\Phi\Delta H = -65.8$ kJ/mol) during this reaction by the standard method reported before.[2,9−10] Under the same condition, we measured the photoacoustic signal and determined the volume change of the reaction to be $\Phi\Delta V = -2.0$ mL/mol.

We calculated the overall stored enthalpy to be −45.2 kJ/mol and the overall volume change −2.65 mL/mol at $T = 274.8$ K and pH = 7 from the data reported by Abbruzzetti et al.[8] These values are close to the overall stored enthalpy and the volume changes determined from the TG signal at 274.8 K (−65.8 kJ/mol and −2.0 mL/mol, respectively). Therefore, the dynamics we observed here by the TG method is considered to be the same one studied before by the PA method. We are now in a position to consider the origin of the enthalpy change and the volume change induced by NBA photodecomposition with Mb.

It may be instructive to consider what TG signal we expect, if the released proton from NBA changes the conformation and the molecular volume of Mb. Since the volume change induces the refractive index change, the species grating signal of Mb should appear and it must decay with a rate constant of $D_{Mb}q^2$. However, contrary to this expectation, the TG signal that decays with the time constant $D_{Mb}q^2$ was not observed after the photoexcitation of NBA with Mb and 0.2 M GdnHCl. This result indicates that the conformational change of Mb induced by the pH jump does not occur in the presence of GdnHCl.

It is interesting to note that the TG signal of NBA + Mb in aqueous solution shows the species grating signal due to Mb. This fact may lead a suggestion that the molecular volume of Mb changes by the pH jump reaction of NBA in aqueous solution. However, we do not think this is a correct interpretation because the observed TG signal shows an additional component, D_I besides the component due to Mb. The presence of this intermediate component indicates that the species grating signal due to Mb appears not because of the conformational change but because Cl^- is diffusing with Mb after the NBA reaction.

To further confirm that the observed grating signal due to Mb is not caused by the conformational change of the protein induced by the pH change, we measured the TG signal in acidic solutions (pH < 4). Although the pH jump cannot be effective in such an acidic solution, we observed a TG signal very similar to that in the aqueous solution (Figure 4a). This result indicates

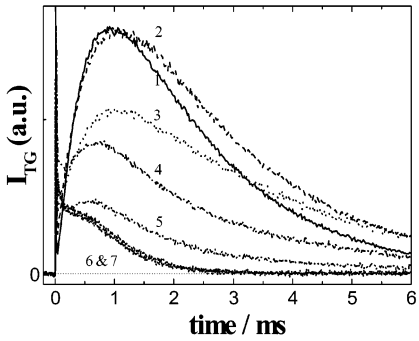

Figure 5. Time profile of the TG signal of NBA + Mb aqueous solution as a function of the concentration of NaCl (1, 0 mM; 2, 1 mM; 3, 2 mM; 4, 3 mM; 5, 5 mM; 6, 50 mM; 7, 100 mM).

that the conformational change is not essential for creating the grating signal. The observed enthalpy change and volume change may reflect the chemical reaction of NBA and subsequent reaction between the NBA reaction product or intermediates and an anion such as Cl^-.

Considering the pH jump reaction in the NBA + Mb aqueous solutions, residues of Mb with a negative charge are protonated by the photoreleased proton, and consequently protein becomes more positively charged. Thus, the positively charged protein can interact with the negatively charged electrolyte such as Cl^- through an electrostatic force. To further confirm the electrostatic interaction between the anion and Mb, we investigate the effect of the electrolyte on the TG signal of NBA + Mb. As shown in Figure 5, upon the addition of NaCl, the strong TG signal observed in NBA + Mb aqueous solutions became gradually weak and was dramatically changed at the high NaCl concentration (>5 mM). It is interesting to note that the TG signal observed at the high NaCl concentration (>5 mM) is very similar to that of NBA in the presence of some electrolytes shown in Figure 2. These results indicate that the interaction between Cl^- and Mb suggested above is inhibited by addition of the electrolyte. Since the surface of the protein is covered with weak acidic and basic groups, the electrostatic shielding of the protein charge induced by the electrolyte leads to the decrease of the interaction between Cl^- and Mb.

5. Conclusions

Photochemical reaction of NBA in various electrolyte solutions as well as in a solution with apomyoglobin is investigated by using the time-resolved TG method. We can show that the diffusion coefficient is a useful property for identifying chemical species involved in chemical reactions even in a case in which optical absorption is negligible. The TG signal of NBA was dramatically changed by adding electrolytes such as Cl^- or Br^-, and the diffusion of the anion such as Cl^- or Br^- was observed as the grating signal. These results indicate that the anion was involved in the NBA photoreaction process. It was found that the time profile of the grating signal also depends on the addition of GdnHCl or Mb. The diffusion of an electrolyte and Mb observed in the NBA + Mb aqueous solutions is due to the interaction between the anion and Mb associated with the NBA reaction rather than the conformational change of Mb induced by the pH jump. One of important messages from this study is that one has to be careful with the reaction of NBA product when experimental results utilizing caged compounds are analyzed.

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