Excited State Intramolecular Proton Transfer and Physical Properties of 7-Hydroxyquinoline

Wee-Kyeong Kang†, Sung-June Cho†, Minyung Lee, Dong-Ho Kim, Ryong Ryoo†, Kyung-Hoon Jung†, and Du-Jeon Jang*,‡

Spectroscopy Laboratory, Korea Research Institute of Standards and Science, Tajon 305-606
†Center for Molecular Science and Department of Chemistry, Korea Advanced Institute of Science and Technology, Tajeon 305-701
Received August 30, 1991

The excited state intramolecular proton transfer and physical properties of 7-hydroxyquinoline are studied in various solutions and heterogeneous systems by measuring steady state and time-resolved fluorescence, reflection and NMR spectra. Proton transfer is observed only in protic solvents owing to its requirement of hydrogen-bonded solvent bridge for proton relay transfer. The activation energies of the proton transfer are 2.3 and 5.4 kJ/mol in CH_3OH and in CH_3OD, respectively. Dimers of normal molecules are stable in microcrystalline powder form and undergo an extremely fast concerted double proton transfer upon absorption of a photon, consequently forming dimers of tautomer molecules. In the supercage of zeolite NaY, its tautomeric form is stable in the ground state and does not show any proton transfer.

Introduction

Proton transfer reactions are among the simplest chemical reactions. Nevertheless they have provided us a vast amount of information for equilibria, kinetics, isotope effects, free energy relationship, etc, compared with any other class of reactions since Arrhenius's acid-base definition. Since 1924[1] the rate constants of proton transfer reactions have been known to be correlated with the equilibrium constants, which can be different by many orders of magnitude between excited and ground states of molecules[2,3]. In the case where functional groups with opposite *pK* tendencies in excited states occupy nearby sites within one molecule, the proton may transfer from one group to the other upon absorption of a photon[4], generating an excited state tautomer, which relaxes to the ground state then undergoes back proton transfer, as depicted in the Figure 1. A variety of molecular systems have been studied extensively for excited state intramolecualr proton transfer recently[5-11].

Kasha[5] suggested that the phenomena of excited state intramolecular proton can be distinguished into four classes according to their molecular mechanisms. Intrinsic intramolecular proton transfer, *e.g.* 3-hydroxyflavone[12] and 2-hydroxy-4,5-benzotropone[13], involves ultrafast proton transfer across an intramolecular hydrogen bond. Concerted biprotic transfer, *e.g.* 7-azaindole dimers and solvates[14], involves cooperative double proton transfer in a cyclic complex. Static and dynamic catalysis of proton transfer, *e.g.* lumichrome[15], involves strong catalysis in doubly hydrogen-bounded acetic acid complexes. Proton relay transfer, *e.g.* 7-hydroxyquinoline (7HQ, I in the Scheme 1)[16-21] and 3-hydroxyxanthone[22,23], is suggested as involving mutiproton-bridged solvates. Proton relay transfer may serve as experimental model for proton relays and proton pumps for transport of protons across membranes in biological systems[24-26]. In the present paper we report a study of the excited state intramolecualr proton transfer of 7HQ in various solutions and heterogeneous systems by observing steady state and time-resolved fluorescence spectra as an attempt to establish a mechanism for proton relays and proton pumps in biological systems.

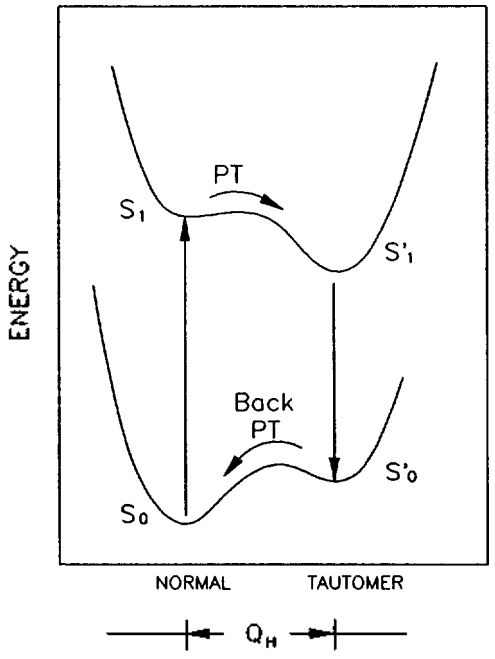
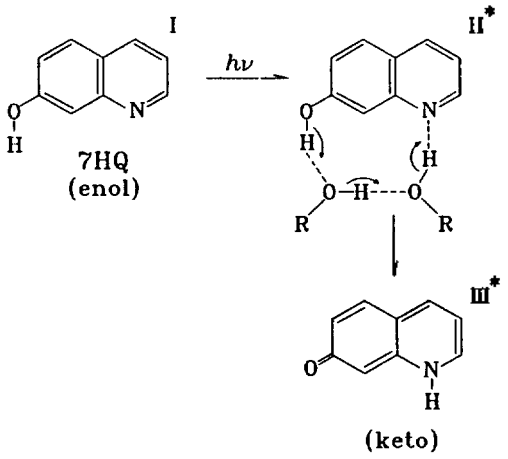


Figure 1. Intramolecular proton transfer double well potential, with Q_H as the proton transfer coordinate. Excited state intramolecular proton transfer follows the photoexcitation of molecules by dramatic changes of acidity and basicity of two adjacent functional group. Then back proton transfer in the ground state follows the relaxation of tautomer molecules.

Mason *et al.*[16] observed that the OH group of 7HQ and 6-hydroxyquinoline is mor acidic and the ring nitrogen atom

* To whom correspondence should be addressed.
‡ Present address: Department of Chemistry, Seoul National University, Seoul 151-742



Scheme 1.

is more basic in the excited state than in the ground state. Excited state proton transfer was first reported in a methanol solution of 7HQ in 1982[17] and the participation of solvent molecules was found to be important in the proton transfer of 7HQ[18]. 7HQ requires two protic solvent molecules to undergo the excited state proton transfer and the involved solvent molecules are considered to act separately at the proton donor and acceptor sites[21], as illustrated in the Scheme 1. 7HQ has been reported[21] to show a remarkably slow back proton transfer time, 3.5 μs in CH_3OH, to S_0 state from S'_0 state and an extraordinarily large deuterium isotope effect, 30 μs in CH_3OD. This could represent more than two proton relay case of intramolecular proton transfer in the ground state.

It would be interesting in the present work to compare the nature of excited state intramolecular proton transfer of 7HQ in various solutions with that in other heterogeneous systems. Adsorbed 7HQ in zeolite might be particularly interesting since the 7HQ in zeolite can be homogeneously distributed as isolated molecules within the supercage of NaY zeolite with the size of 1.3 nm. Its proton transfer characteristics might stress the role of protic solvent in the process.

Experimental

Materials. The compounds of 7HQ and 8-hydroxyquinoline (8HQ) purchased from Aldrich were purified by vacuum sublimation. Spectral grade alcoholic solvents were used without further purification. Ethanol-free chloroform, obtained by shaking spectral grade chloroform with concentrated sulfuric acid, was washed with water, dried over calcium chloride and then slowly distilled in a fractionating column under nitrogen atmosphere. Deutrated 7HQ (7DQ) in CH_3OD was prepared by equilibrating 7HQ in 99% CH_3OD three times. The typical concentration of 7HQ in solutions was 1×10^{-4} M. The solution temperature was controlled using a cold finger of an Air Products 202E open cycle helium displex. High-purity zeolite was synthesized, washed with doubly distilled hot water and dried in a vacuum oven. The structure was identified by using the methods of X-ray diffraction and ^{129}Xe NMR. Unit cell formula of this sample after complete dehydration at 670 K was determined to be $Na_{56}(AlO_2)_{56}(SiO_2)_{136}\cdot 250H_2O$ by elemental analysis. A Pyrex test tube containing 7HQ (or 8HQ) and a tube containing NaY zeolite powder were jointed with a vacuum port. The 7HQ and NaY samples were maintained at 273 K and 720 K under vacuum for 2 h, respectively. The vacuum port was then sealed off by flame. 7HQ molecules were introduced into the supercage of the NaY zeolite by sublimating 7HQ with placing the two sample tubes at a certain temperature (520 K hereafter if preparation temperature is not mentioned) which were jointed under vacuum. After sublimating 7 HQ for 2 h, the sample was slowly cooled down to maintain a uniform distribution of the organic molecules in the zeolite sample. Then the sample was transferred into a quartz cell or a specially designed NMR tube under a dry N_2 atmosphere.

Steady State Measurements. Static fluorescence spectra were obtained using an Aminco-Bowman Spectrophotometer. All the fluorescence spectra reported here are not corrected for the variation of the detector sensitivity as a function of wavelength. Reflection spectra were measured using a Shimadzu UV-VIS-NIR Recording Spectrophotometer. The reference material used for the reflection measurements was magnesium oxide powder. For ^{129}Xe-NMR study, xenon gas (Matheson, 99.995%) of desired pressure was equilibrated with the sample at 296 K through stopcocks on the NMR tube. ^{129}Xe-NMR spectrum was obtained by a Bruker AM-300 instrument operating at 83.0 MHz. Neither the sample spinning nor the field locking was employed. Each spectrum was obtained after accumulating a 90°-pulse transient more than 100 times. The chemical shift was referenced to the xenon in bulk gas phase extrapolated to zero pressure. The chemical shift variation and the NMR line splitting were used to obtain the information on the distribution of 7HQ in zeolite.

Fluorescence Kinetic Measurements. A Coherent Antares Nd : YAG laser was actively mode-locked at 76 MHz and frequency-doubled by a KTP crystal to synchronously pump a dual-jet dye laser. The cavity-dumped pulse from the dye laser had ~2 ps pulse width, ~120 mW average power at 3.8 MHz dumping rate and 560-607 nm tunability when Rh6G for gain dye and DODCI for saturable absorber dye were used. In order to excite the 7HQ, the dye laser pulse was frequency-doubled using a KDP crystal. Wavelength-selected fluorescence from 7HQ using a Jobin-Yvon 0.2 m spectrometer and a combination of optical filters was focused to a Hamamatsu R928 photomultiplier tube. Fluorescence kinetics were measured by a time-correlated single photon counting system. All the standard electronics for the time-correlated single photon counting system were obtained from EG & G Ortec. The instrument response function was determined by measuring light scatter from pure *n*-pentane and it was typically 700 ps.

Results and Discussion

Figure 2 shows the fluorescence spectra of 7HQ in chloroform and in methanol. The methanol solution shows dual fluorescence with peaks at 382 nm (un band) and at 520 nm (green band) while the chloroform solution exhibits the uv fluorescence band only. The uv band is due to the normal fluorescence and the green band is attributed to the tauto-

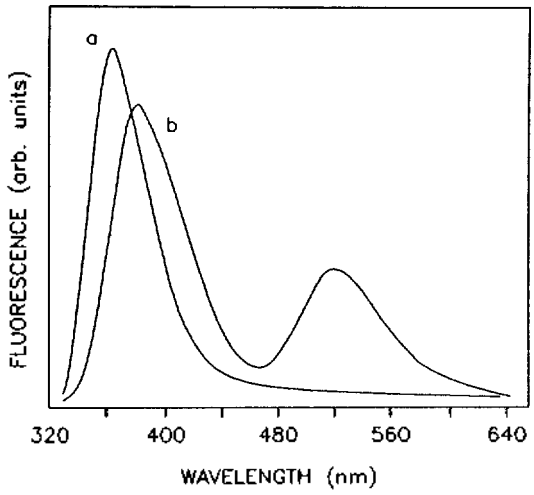


Figure 2. Fluorescence spectra of 7HQ in chloroform (a) and in methanol (b). The methanol solution shows the normal (with the peak at 382 nm) and tautomer fluorescence (with the peak at 520 nm) while the chloroform solution shows the normal fluorescence (with the peak at 363 nm) only.

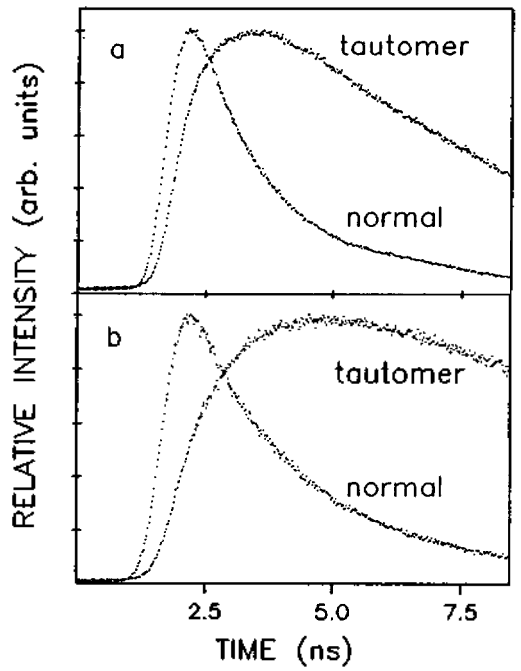


Figure 3. Normal and tautomer fluorescence rise and decay kinetic at 180 K of 7HQ in CH_3OH (a) and 7DQ in CH_3OD (b). The deconvoluted rise and decay time constants and relative amplitudes are listed in the Table 1.

mer fluorescence, as observed previously[18]. The large red shift of the normal fluorescence in methanol compared to the fluorescence in chloroform indicates that the lowest transition is π–π^* transition. Aromatic ring nitrogens are typically more basic in the π–π^* excited state than in the ground state and the reverse is true for aromatic alcohols[27]. As a result, the proton in the OH group of 7HQ in methanol solution transfers to the nitrogen upon absorption of a photon, forming excited state keto form (III*), the tautomer form

Table 1. Fluorescence Decay and Rise Times of 7HQ

Solvent	Normal	Tautomer		T (K)	E_a (kJ/mol)
	Decay (ns)	Rise (ns)	Decay (ns)		
CH_3OH	0.2 (70%)[a]	0.2	2.9	RT	
	1.5 (30%)[a]				
	0.9 (80%)[a]	0.9	5.4	180	2.3[b]
	4.5 (20%)[a]				
CH_3OD	0.3 (60%)[a]	0.3	5.6	RT	5.4[b]
	1.9 (40%)[a]				
	1.6 (70%)[a]	1.4	11	180	
	4.2 (30%)[a]				
CH_3Cl	0.5			RT	
Powder	$\ll$0.1	$\ll$0.1	0.2	RT	
Zeolite			2.7 (40%)[a]	RT	
(NaY)			8.0 (60%)[a]		

[a] The percentages in parentheses indicate relative amplitudes for respective decay components. [b] The activation energies were determined using the temperature dependent rise times measured at every 20 K from 180 K to 300 K although this table shows data only at two temperatures for a better simple comparison.

of 7HQ, as shown in the Scheme 1. In ethanol-free chloroform solution, 7HQ does not show the tautomer fluorescence, indicating that excited state proton transfer cannot undergo without hydrogen-bonded solvent bridge.

The fluorescence rise and decay kinetics in methanol solution, illustrated in the Figure 3 and Table 1, show that the fast decay time of the biphasic normal fluorescence is the same as the rise time of the tautomer fluorescece. This indicates that the major quenching channel of the normal fluorescence decay is due to the proton transfer. The normal molecules showing the slow decay component of the biphasic normal fluorescence do not seem to undergo excited state proton transfer within the lifetime of the excited state since the tautomer fluorescence does not show the corresponding rise component. Figure 3 also demonstrates that the excited state proton transfer is slower in CH_3OD than in CH_3OH. The proton transfer time at room temperature was observed to be 0.2 ns in CH_3OH and 0.3 ns in CH_3OD, respectively. This isotope effect is relatively small cmpared to a reported isotope effect[21] of the back proton transfer in the ground states. Itoh *et al.*[21] have reported that the back proton transfer times in the ground state are 3.5 μs in CH_3OH and 30 μs in CH_3OD, respectively. This difference in the isotope effect between the proton transfers in the excited and ground states suggests that more solvent molecules might be involved in the hydrogen-bonded solvent bridge of the proton transfer in the ground state.

The activation energy of excited state proton transfer was determined using the temperature dependent rise times of the tautomer fluorescence measured at every 20 K from 180 K to 300 K and it was found to be 2.3 and 5.4 kJ/mol for 7HQ and 7DQ, respectively. It is worth while comparing these activation energies with those in the ground state, 18 and 23 kJ/mol for 7HQ and 7DQ, respectively[21]. The activation energy in the excited state is much smaller than that in the ground state, as expected based on the rate difference

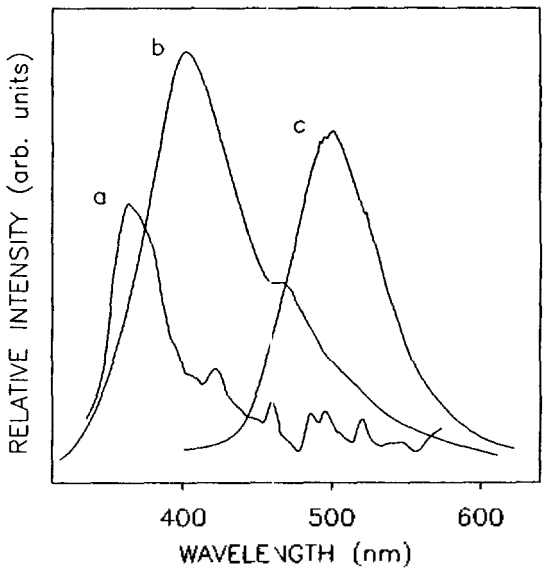


Figure 4. Fluorescence spectra of 7HQ in methanol at 77 K (a), powder form (b) and zeolite NaY (c). The fluorescence peak wavelengths are 363, 403 and 502 nm for 7HQ in methanol at 77 K (a), powder form (b) and zeolite NaY (c), respectively. The powder form shows also a hump at 470 nm.

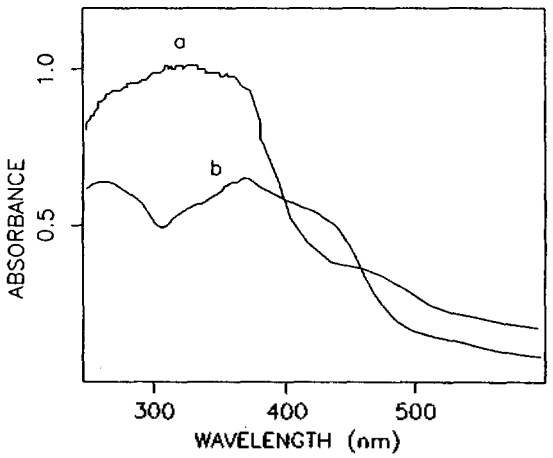


Figure 5. Reflection spectra of 7HQ in powder form (a) and in zeolite NaY (b). Reference material was MgO powder. Dimers of normal molecules are stable in powder form and tautomer molecules are stable in the supercage of zeolite NaY.

of proton transfer between the ground and excited states. It is interesting to note that the activation energy difference between 7HQ and 7DQ in the ground state is very similar to that in the excited state. This might indicate that the activation energy differences in both ground and excited states are due, at least partially, to the vibrational zero-point energy differences between 7HQ and 7DQ in both states. The fluorescence spectrum of the methanol solution at liquid nitrogen temperature shows the normal fluorescence only, as shown in the Figure 4(a). This suggests that the excited state proton transfer may not undergo in a rigid glassy system. In a rigid glassy system, solvent reorganization to make the necessary solvent bridge for proton transfer may not occur within the lifetime of the excited state. Nevertheless, proton transfer could occur *via* a pre-existed solvent bridge although the fraction of molecules with a pre-existed solvent bridge might be small. No observation of the excited state proton transfer at liquid nitrogen temperature indicates that proton transfer cannot occur within the lifetime of the excited state at this low temperature even for the molecules with a pre-existed solvent bridge. It is interesting to notice that the fluorescence peak wavelength of methanol solution at liquid nitrogen temperature shifts to the peak wavelength of chloroform solution at room temperature.

The vacuum sublimated powder form of 7HQ emits fluorescence with a peak at 502 nm and a hump at 470 nm as shown in the Figure 4(b). The emission at 400 nm exhibits an extremely fast decay time and the emission at 500 nm with the hump shows a 0.2 ns decay component (3% in relative amplitude) besides the extremely fast decay component, as listed in the Table 1. From the foregoing discussion, we suggest that the emission band showing the maximum at 403 nm is attributed to fluorescence from normal excimer molecules and that the emission band showing the maximum at ~500 nm and the hump at 470 nm is due to fluorescence from tautomer excimer molecules. In microcrystalline powder form, the proton of the OH group in a molecule and the nitrogen atom of the neighboring molecule may form a direct cyclic hydrogen bond, resulting the formation of a ground state stable dimer. Upon absorption of a photon, the excimer of a normal molecule and an excited state normal molecule undergo an extremely rapid concerted double proton transfer *via* the pre-existed hydrogen bond, resulting in the formation of an excimer of tautomer and excited state tautomer molecules. Since the releasing proton and the accepting nitrogen are directly in contact *via* hydrogen bond, this double proton transfer is expected to be extremely rapid as intrinsic intramolecular proton transfer in nature. This proton transfer time is too fast to measure with the current temporal resolution. The relatively small (3%) amplitude of the tautomer excimer fluorescence decay indicates that other extremely fast nonradiative relaxation processes for the normal excimer molecules are predominant over the double proton transfer. Since the rise time of the tautomer excimer fluorescence is too fast to be resolved with the current temporal resolution, there is a possibility that the ground state stable tautomer molecules or the tautomer dimers exist in equilibrium with the ground state stable normal molecules or the normal dimers and that both ground state stable species form excimers immediately upon absorption of a photon. The reflection spectrum of 7HQ powder form in the Figure 5(a) shows that tautomer molecules or tautomer dimers are not stable but normal dimers are stable in the ground state. The reflection spectrum is spectrally located between the absorption spectrum of normal molecules and the transient absorption spectrum of tautomer molecules, spectrally similar to a typical dimer absorption.

Recent studies[28,29] on adsorbed organic molecules in zeolite supercage using ^{129}Xe-NMR showed that the chemical shift of adsorbed xenon can increase due to the interaction between the adsorbed xenon and the organic molecule within the supercage. We also investigated the distribution of hydroxyquinolines on NaY zeolite by using the ^{129}Xe-NMR technique. Figure 6 shows ^{129}Xe-NMR spectra obtained from NaY zeolite after the adsorption of 7HQ and 8HQ at various

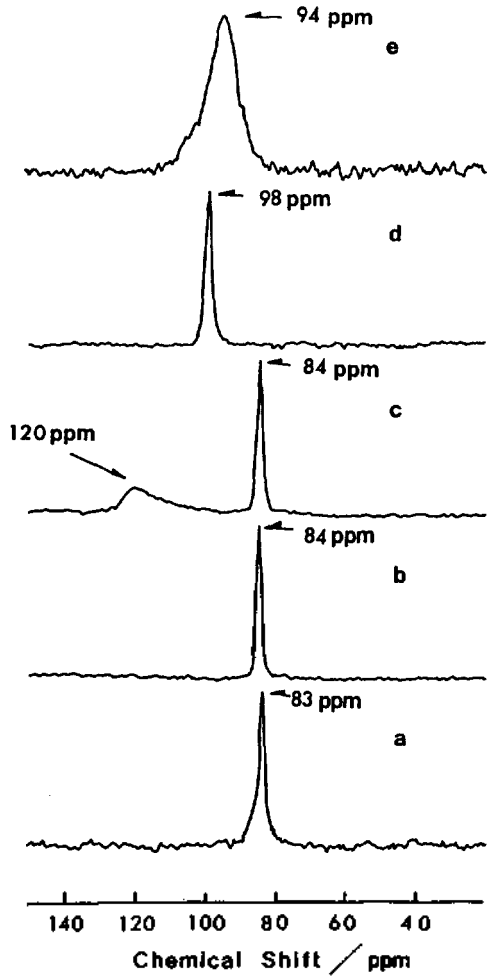


Figure 6. ^{129}Xe-NMR spectra of adsorbed xenon for NaY (a), 7HQ in NaY prepared at 520 K (b), 8HQ in NaY prepared at 520 K (c), 8HQ in NaY prepared at 600 K (d) and 7HQ in NaY prepared at 600 K (e), respectively. The supercage can contain only one HQ molecule at most. The NMR spectra were obtained in 400 torr xenon at 296 K.

temperatures. Spectrum(b), which was obtained after adsorption of 7HQ molecule in supercage at 520 K, does not show any significant difference from the spectrum(a) obtained from NaY zeolite under the same experimental conditions. It therefore seems that 7HQ does not enter the supercage by heating at 520 K. On the other hand, the spectrum(c) which was obtained after adsorption of 8HQ under the same condition shows the appearance of two well-resolved NMR lines indicating that 8HQ enters to adsorb in the supercage: one has the same chemical shift as NaY zeolite and the other has an increased chemical shift. Such a line splitting in ^{129}Xe-NMR at room temperature with microcrystalline (1 μm size) NaY zeolite is due to macroscopically heterogeneous distribution of the adsorbed species[30]. A single Lorentzian NMR peak with a larger chemical shift in the spectrum(d) indicates that 8HQ molecules are uniformly distributed within the micropores of zeolite at 600 K. Compared with the result from 8HQ, a significant line broadening in the spectrum(e) shows that the distribution of 7HQ under this condition is less uniform. Such a difficulty in the uniform distribution of 7HQ may come from a diffusional hindrance. 7HQ cannot form intramolecular hydrogen bonding while 8HQ does. Consequently, 7HQ can dimerize or bind to the outside surface of zeolite through intermolecular hydrogen bonding. 7HQ diffuse into the supercage at higher temperature and less uniformly, compared to 8HQ. According to Itoh *et al.*[21] the 1 : 2 stoichiometric hydrogen bonding complex of 7HQ and methanol undergoes proton transfer. The OH group releases a proton to a solvent molecule and the nitrogen atom abstracts a proton from solvent, producing a transient zwitterionic form[21]. We have investigated if 7HQ adsorbed inside the supercage of the NaY zeolite also undergoes a similar proton transfer reaction since the zeolite has oxygen atoms and hydroxylic groups on the supercage wall. The emission spectrum of 7HQ in the supercage of NaY zeolite, displayed in the Figure 4(c), shows tautomer fluorescence only, indicating that entire excited state molecules undergo proton transfer or that tautomer molecules are stable in the ground state. The reflection spectrum of the Figure 5(b) demonstrates that the tautomer molecules of keto form are stabler than the normal molecules of enol form in the ground state. Fluorescence kinetic measurements show a biphasic decay of 2.7 and 8.0 ns time constants, probably due to heterogeneous characters of zeolite surfaces.

In conclusion, reflection, fluorescence and NMR spectroscopies and time-resolved fluorescence spectroscopy have been performed to investigate the excited state intramolecular proton transfer and physical properties of 7HQ in various solutions and heterogeneous systems. Methanol solutions show dual normal and tautomer fluorescence, while chloroform solution show only the normal fluorescence. Proton transfer is observed only in protic solvents owing to its requirement of hydrogen-bonded solvent bridge for proton relay transfer. The activation energies of the proton transfer are 2.3 and 5.4 kJ/mol in CH_3OH and CH_3OD, respectively. Dimers of normal molecules are stable in the microcrystalline powder form of 7HQ and undergo an extremely fast concerted double proton transfer upon absorption of a photon, consequently forming dimers of tautomer molecules. However, the relatively small amplitude of the tautomer excimer fluorescence indicate that other extremely fast nonradiative relaxation processes are predominant over the double proton transfer. In the supercage of zeolite NaY, the tautomeric form is stable in the ground state and does not show any proton transfer. 8HQ, compared to 7HQ, diffuses into the supercage of zeolite at lower temperature and more uniformly.

Acknowledgements. This work was done with financial assistance from the Ministry of Science and Technology and from the Center for Molecular Science under the program of Science Research Centers by the Korea Science and Engineering Foundation.

References

1. J. N. Bronsted and K. Pedersen, *Z. Physik. Chem. Stoichiom. Verwanschaftsl.*, **108**, 185 (1924).
2. T. Forster, *Z. Elektrochem.*, **54**, 43 (1950).
3. A. Weller, *Z. Electrochem.*, **60**, 1144 (1956).
4. A. Weller, *Progr. React. Kinet.*, **1**, 188 (1961).
5. M. Kasha, *J. Chem. Soc. Faraday Trans. 2*, **82**, 2379 (1986).

6. F. Laermer, T. Elsaesser, and W. Kaiser, *Chem. Phys. Lett.*, **148**, 119 (1988).
7. G. A. Brucker and D. F. Kelley, *J. Chem. Phys.*, **90**, 5243 (1989).
8. P. F. Barbara, P. K. Walsh, and L. F. Brus, *J. Phys. Chem.*, **93**, 29 (1989).
9. J. Catalan, F. Fabero, M. S. Guijarro, R. M. Claramunt, M. D. S. Maria, M. C. Foces-Foces, F. H. Cano, J. Elguero, and R. Sastre, *J. Am. Chem. Soc.*, **112**, 747 (1990).
10. M. Itoch, Y. Fujiwara, M. Matsudo, A. Higashikata, and K. Tokumura, *J. Phys. Chem.*, **94**, 8146 (1990).
11. D.-J. Jang, *Bull. Korean Chem. Soc.*, **12**, 441 (1991).
12. D. MaMorrow and M. Kasha, *J. Phys. Chem.*, **88**, 2235 (1984).
13. D.-J. Jang, G. A. Brucker, and D. F. Kelley, *J. Phys. Chem.*, **90**, 6808 (1986).
14. C. A. Taylor, M. A. El-Bayoumi, and M. Kasha, *Proc. Natl Acad. Sci. USA*, **63**, 253 (1969).
15. P.-S. Song, M. Sun, and A. Koziolawa, *J. Am. Chem. Soc.*, **96**, 4319 (1974).
16. S. F. Mason, J. Philp, and B. E. Smith, *J. Chem. Soc.*, 3051 (1968).
17. P. J. Thistlethwaite, and P. J. Corkill, *Chem. Phys. Lett.*, **85**, 317 (1982).
18. P. J. Thistlethwaite, *Chem. Phys. Lett.*, **96**, 509 (1983).
19. M. Itoch, T. Adachi, and K.Tokumura, *J. Am. Chem. Soc.*, **105**, 4828 (1983).
20. K. Tokumura and M. Itoch, *J. Phys. Chem.*, **88**, 3921 (1984).
21. M. Itoch, T. Adachi, and K. Tokumura, *J. Am. Chem. Soc.*, **106**, 850 (1984).
22. R. Schipfer, O. S. Wolfbeis, and A. Knierzinger, *J. Chem. Soc. Perkin Trans, 2, 1443 (1981).*
23. *M. Itoh, N. Yoshida, and M. Takashima, J. Am. Chem. Soc.*, **107**, 4819 (1985).
24. A. Lewis, M. A. Marcus, B. Ehrenberg, and H. Crespi, *Proc. Natl. Acad. Sci. USA*, **75**, 4642 (1978).
25. D. A. Marvin, *FEBS*, **156**, 1 (1983).
26. D.-J. Jang, M. A. El-Sayed, L. J. Stern, T. Mogi, and H. G. Khorana, *Proc. Natl. Acad. Sci. USA*, **87**, 4103 (1990).
27. J. F. Ireland and P. A. H. Wyatt, *Advances in Physical Organic Chemistry*, Vol. 12, (Academic, London, 1976), p. 131.
28. B. F. Chmelka, J. G. Pearson, S. -B. Liu, R. Ryoo, L. C. de Menorval, and A. Pines, *J. Phys. Chem.*, **95**, 303 (1991).
29. L. C. de Menorval, D. Raftery, S. -B. Liu, K. Takegoshi, R. Ryoo, and A. Pines, *J. Phys. Chem.*, **94**, 27 (1990).
30. R. Ryoo, C. Pak and B. F. Chmelka, *Zeolites*, **10**, 791 (1990).

Microbial BOD Sensor Using *Hansenula anomala*

Gwon-Shik Ihn, Kyung-Ho Park†, Un-Hua Pek†, and Moo-Jeong Sohn*

Department of Chemistry, Keimyung University, Taegu 704-200
**Doosan Research Laboratories Yoido, P. O. Box 80.* *Received October 5, 1991*

A microbial sensor for BOD (Biochemical Oxygen Demand) measurement has been developed by immobilizing *Hansenula anomala* in a polyacrylamide gel. The optimum pH and temperature for BOD measurement using this sensor were pH 7.0 and 30℃, respectively. The response time was 30 min. A linear relationship was observed between the potential and the concentration below 44 ppm BOD. The potential was reproducible within ± 9% of the relative error when a sample solution containing 20 mg/*l* of glucose and 20 mg/*l* of glutamic acid was employed. The effect of various compounds on BOD estimation was also examined. The potential output of the sensor was almost constant for 30 days. The relative error in BOD estimation was within ± 10%.

Introduction

Biochemical Oxygen Demand (BOD) is one of the most widely used and important tests in the measurement of the organic pollution in waste waters, effluents, and polluted waters. The 5-day BOD test has remained a standard pollution monitoring tool since 1936[1]. In the 5-day BOD test, the bottle size, incubation temperature (20℃) and incubation period (5 days) are all specified as well as, furthermore, the skill of operators is also required. Therefore, because the 5-day BOD test is too long and complex for use in process control, rapid and reproducible methods are desirable. In an effort to develop a shorter test for a given sample, a bioelectrochemical sensor consisting of microorganisms immobilized and dissolved oxygen electrode has been developed.

The first BOD sensor was described by Karube *et al.*[2], in which *Clostridium butyricum*-collagen membrane and oxygen probe was used. Furthermore, various microbial BOD sensors using microorganisms, such as *Trichosporon cutaneum*[3,4], *Hansenula anomala*[5], *Pseudomonas* sp.[6], *Escherichia coli*[7], *Bacillus subtilis*[4], and thermophilic bacteria[8] have been developed by many authors.

In this paper, a microbial BOD sensor consisting of immobilized *Hansenula anomala* in a polyacrylamide gel and an