Hydration of N-Monosubstituted Amides in the Binary Solvents Dioxane-D₂O and Dioxane-H₂O

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The molecular-level structure of hydrated N-monosubstituted amides in aqueous media has been studied by measuring the infrared absorption spectra due to the C=O stretching (or amide I band) of a series of N-monosubstituted amides (RCONHR') in dioxane-D₂O and dioxane-H₂O mixtures. Three kinds of hydrated species of the secondary amides have been identified, namely monohydrated amide (II) {at the amide oxygen}, dihydrated amide (III) {monohydrated at both the amide oxygen and the amide NH}, and trihydrated amide (IV) {dihydrated at the amide oxygen and monohydrated at the amide NH}. With increasing water content, the relative amounts of the hydrated amides increases in the order II < III < IV. In pure heavy water, all of the amide molecules are hydrated, and two or three hydration states are in equilibrium. The degree of hydration is little influenced by the electronic character of the substituent R, whereas it is considerably influenced by the hydrophobic character of the substituent R'. As to the H/D isotope effect, the hydrogen bonding strength of C=O···HOH is stronger than that of C=O...DOD for N-alkylbenzamides.

Introduction

Knowledge of the hydrogen bonding interaction between amides and water molecules in aqueous media is of particular interest as a basis for the extention to more complicated cases of proteins and peptides in biological systems,¹ and hence a number of both theoretical²⁻⁶ and experimental⁷⁻¹¹ studies have been published so far. Most experimental studies dealt with macroscopic thermochemical quantities of solution, and data relating to the molecular-level structure of hydrated amides in solution are very limited.

Previously, one of the present authors (M.K.) found that infrared absorption due to the C=O stretching, amide I band $\nu_{C=O}$ of primary amides (RCONHR', R' = H) dissolved in dioxanewater (H₂O, D₂O) mixtures exhibited a very remarkable red shift with an increase of water content in the medium.¹² The spectral change was attributed to the change in the hydration states of amides. It has been concluded that (1) amides are hydrated in dioxane-water mixtures, and the averaged degree of hydration changes in proportion to the content of water; (2) the degree of hydration is influenced mainly by a steric effect rather than the polar effect of the substituent R and is larger in aliphatic amides than in aromatic amides; (3) for the deuterium isotope effects, stronger hydration exists in D_2O mixtures than in H_2O mixtures. However, elucidation of the chemical form of hydrated amides remained as one of the important tasks to be studied.

The carbonyl oxygen of amides acts as a proton acceptor, while the amide NH acts as a proton donor. Therefore, two types of hydration are possible for primary and secondary amides, and both types coexist in binary medium of high water content. Actually, thermochemical data have shown that, in aqueous solution, a

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primary or secondary amide molecule is bound by three water molecules: two at the amide oxygen and one at the NH group, as illustrated in structure A.¹¹ The bond length variation rule¹³



suggests that the $\nu_{C=0}$ frequency shift caused by hydration is greater for amides hydrated at the carbonyl oxygen than for those hydrated at the NH proton. In fact, the spectral data of Nmethylacetamide (NMAA) embedded in an argon matrix have shown that, in the linear dimer of NMAA, the $\nu_{C=0}$ frequency of the bonded C=O {(ii) in structure B} shifts to the low-frequency



side by 23 cm⁻¹ from that in monomeric NMAA, whereas that of the free C=O $\{(i) \text{ in } B\}$ shifts by 11 cm^{-1.14} It is anticipated that, by analyzing the change in the spectral profile of the amide I band with the composition of the binary solvents (mole fraction of water, X_w), we are able to elucidate both the chemical form and the hydration number of the hydrated amides.

In order to elucidate the chemical form and hydration number of hydrated amides, and also to make the effects of the substituent R' on the hydration clearer, we measured the infrared spectra of the following series of N-substituted amides in binary solvents of dioxane-water (H_2O, D_2O) with various compositions: Nmethylbenzamide (NMBA), N-methyl-p-chlorobenzamide (NMClBA), N-methyl-p-methoxybenzamide (NMMeOBA), N-ethylbenzamide (NEBA), and N-phenylacetamide (NPhAA). The obtained absorbance profiles of the amide I bands were analyzed.

Experimental Section

Dioxane of a spectroscopic grade, purchased from Wako Pure Chemical Industry Ltd., was refluxed with metallic sodium for

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	structural	mp, °C			
	formula		ref	solvents	
NMBA	с _е н ₅ —С—N< ^H	78	78ª	ethanol-water (1:2 in vol)	
NMCIBA	ρ-cι−c ₆ H ₄ −C−N< ^H _{CH3}	158.5	161 ^b	dioxane-water (1:1 in vol)	
NMMeOBA	р-сн ₃ 0-с ₆ н ₄ -с-N<	118.7	118°	water	
NEBA	C ₆ H ₅	68.5	68 ^d	water	
NPhAA	сн ₃ —с_ N<	113.6	113–114 ^e		

^aReference 15. ^bReference 16. ^cReference 17. ^dReference 18. ^eReference 19.



Wavenumber/cm⁻¹



several hours and was distilled just before use. The distillate at 101.4 °C was taken. Ion-exchanged water was used after distillation. Dueterium oxide, claimed to be 99.75% pure, was obtained from Merck and used without further purification.

NMBA, NMCIBA, NMMeOBA, and NEBA were synthesized by adding dropwise acid chloride of each of the corresponding compounds into methylamine (40% aqueous solution) and/or ethylamine (70% aqueous solution). The resulting precipitates were purified by recrystallization. NPhAA of guaranteed grade obtained from Merck Co. Ltd., was used without further purification. The structural formulae and the melting points of the amides, along with the solvents used for recrystallization, are summarized in Table I. The N-deuteriated species of these amides



Figure 2. The amide-I band of NMBA in dioxane-water mextures at various mole fractions of water (X_w) : (a) 0, (b) 0.11, (c) 0.14, (d) 0.32, (e) 0.42, (f) 0.54, (g) 0.76, (h) 0.9, (i) 1.0. (D₂O) NMBA-d in dioxane-D₂O; (H₂O) NMBA in dioxane-H₂O.

were obtained by repeated recrystallization of normal species from D₂O, until the NH stretching infrared band intensity decreased to almost 1% of the ND stretching band intensity.

Infrared spectra in the frequency range of 1500–1800 cm⁻¹ were recorded in the linear absorbance scale with a Jasco IR-A 302 spectrophotometer equipped with a data processor, employing a pair of solution cells of 0.025-mm path length with calcium fluoride windows. The mole fraction of the binary mixed solvent covers the whole range in the case of D_2O , while it was limited from 0 to 0.54 in H₂O because of masking by strong absorption due to normal water. The amide concentration was kept as low as 0.05 mol dm⁻³ to lessen the presence of self-associated species, which are negligible in a solution of such low concentration by judging from the absorbance of the bonded band as shown in Figure 1.

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⁽²⁰⁾ Band-shape analysis shows that the band of bonded C==O consists of three components. The components are referred to the carbonyl group in the cyclic dimer, the carbonyl group hydrogen bonding at the carbonyl oxygen in the linear dimer, and the carbonyl group hydrogen bonding not at the carbonyl oxygen but at the adjacent NH group in the linear dimer. Details on self-association of N-substituted amides are now in press in Annu. Rep. Res. Reactor Inst. Kyoto Univ.

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Further details of the experimental procedure are the same given in Ref 12.

Results and Discussion

Figure 2 shows the amide-I band of NMBA in dioxane-water mixtures at various values of X_w . The absorption curve exhibits remarkable and characteristic changes as the amount of water increases, not only in the peak position, but also in the band shape. It changes from a very symmetrical singlet to complicated multipeak profiles. The asymmetric curves seem to be composed of a finite number of symmetric-shaped constituents. Each of them is thought to correspond to one of the different hydration states involved.

The absorption curves in Figure 2 (D₂O) have major peaks at 1663.5, 1645, 1633, and 1617 cm⁻¹. They are referred to as the bands I, II, III, and IV, or components I, II, III, and IV, respectively. It is likely that in the binary solvent system the NMBA-*d* molecule assumes, at least, four different states: namely, unhydrated NMBA-*d* and three differently hydrated forms. The curve for $X_w = 0$ (namely, in pure dioxane) is symmetric with a single peak at 1663.5 cm⁻¹ (band I) and, therefore, is recognized as consisting of only one component. Thus, band I should be assigned to $\nu_{C=0}$ of unhydrated NMBA-*d*. The contribution of band I to the absorption curves decreases with increasing X_w and vanishes in pure D₂O. Hence, all of the amide molecules dissolved in pure water are in hydrated states. From the spectral change with X_w , it is concluded that bands II, III, and IV are associated with hydrated NMBA-*d*'s, and the hydration number must increase in the order of the components II < III < IV.

The frequency differences between component I and II ($\Delta \nu$ -(I–II)), II and III ($\Delta \nu$ (II–III)), and III and IV ($\Delta \nu$ (III–IV)) are 18.5, 12, and 16 cm⁻¹, respectively. Bearing in mind the spectral data for NMAA embedded in an Ar matrix,¹⁴ we assigned band II to NMBA-*d* monohydrated at the amide oxygen (II), band III



to dihydrated NMBA-d (monohydrated both at the amide oxygen and the amide NH (III)), and band IV to trihydrated NMBA-d (dihydrated at the amide oxygen and monohydrated at the amide NH (IV)).

Equilibria 1-3 are considered in the present case.

$$NMBA-d + D_2O \rightleftharpoons II \tag{1}$$

$$II + D_2 O \rightleftharpoons III \tag{2}$$

$$III + D_2 O \rightleftharpoons IV \tag{3}$$

As mentioned above, all of the amide molecules are free from hydration in pure dioxane. With increasing X_w , the relative amount of the free amide decreases and instead there arises first the monohydrated species and thereafter the dihydrated species. For instance, in the media at $X_w = 0.14$ and 0.23, the main chemical forms of NMBA-*d* present are free and monohydrated, while at $X_w = 0.76$ both monohydrated and dihydrated amides become predominant. At more water-rich composition such as $X_w = 0.9$, a considerable fraction of amide molecules are trihydrated. In pure heavy water, NMBA-*d* molecules are di- and/or trihydrated. Spencer reported that primary and secondary amides in pure water were trihydrated as described by the structure A with R and R' = CH₃.¹¹ The present result indicates the presence of both dihydrated and trihydrated amides in pure heavy water, contrary to Spencer's conclusion. At any rate, it should be emphasized that, even in pure heavy water, probably also in pure water, two or more hydration states are in equilibrium.

The amide-I bands of NMClBA, NMMeOBA, NEBA, NPhAA, and their N-deuterio derivatives were also measured in dioxane-water (D_2O , H_2O) mixtures of various compositions. For all the amides investigated a relationship similar to that for NMBA was obtained between the band profile and the composition of the solvent, as shown in Figure 3.

As a measure of the degree of hydration to amide molecules, we introduced previously¹² the weighted mean frequency, $\bar{\nu}_{C=0}$, defined by the equation

$$\bar{\nu}_{C=0} = \sum_{i=1}^{n} P_i \nu_i / \sum_{i=1}^{n} P_i$$

for a particular amide solution, where P_i and ν_i are the percentage contribution and the characteristic C=O stretching frequency of the amide molecule in the *i*th hydration state, respectively. For all the N-substituted amide solutions, the values of $\nu_{C=O}$ were obtained directly from the recorded absorbance curve by seeking the wavenumber at which the area under the curve was divided in half.

For the primary amides, a plot of $\bar{\nu}_{C=0}$ vs. mole fraction of water in the media gave a straight line.¹² Similarly, a good linear relationship between $\bar{\nu}_{C=0}$ and X_w was obtained for the N-substituted amides as shown in Figure 4. The equation expressing the straight line was obtained by the least-squares method. The intercept (ν_0) of the line gives the value of $\bar{\nu}_{C=0}$ in pure dioxane. The slope of the line ($\Delta \nu$) indicates the frequency shift of $\bar{\nu}_{C=0}$ between pure dioxane and pure water. This value is regarded as a measure of the degree of hydration in pure water, and hence the ratio of $\Delta \nu_{H_2O} / \Delta \nu_{D_2O}$ represents the H/D isotope effect of the overall hydration. The values of $\Delta \nu$ and ν_0 and the ratios of $\Delta \nu_{H_2O} / \Delta \nu_{D_2O}$ are listed in Table II.

The value of $\Delta \nu$ includes two factors; the distribution of various hydration states present in pure water and the strength of the NH…OH₂ and C=O…HOH hydrogen bonds. In principle, these two factors are expected to depend on the structure of the substituent groups of R and R'.

First, we discuss the effect of substituent R. In Table II, NMBA, NMMeOBA, and NMClBA show almost the same value of Δv within experimental error for H₂O and D₂O mixed solvents. Similarly, the difference in Δv between benzamide and *p*-methoxybenzamide is not significant. The *p*-methoxyphenyl group (the Hammett σ value = -0.268 for p-CH₃O) is more electron donating and the *p*-chlorophenyl group ($\sigma = +0.227$ for *p*-Cl) is more electron attracting than the unsubstituted phenyl group ($\sigma = O$ for H). It is most likely that the electronic character of the substituent R does not influence the degree of hydration of amides in water. The electron-donating R increasing the electron density at the amide group, promotes C=O...HOH bonding, and suppresses the $NH - OH_2$ bonding. On the contrary, the electronattracting R decreasing the electron density at the amide group, suppresses the C==O--HOH bonding, and promotes the NH--OH2 bonding. The values of $\Delta \nu$ as a measure of the degree of hydration contain contributions from two types of hydration. As a result, the electronic effects of the substituent R on two different hydration sites of the amide molecule appear to be cancelled. As is pointed out in the previous paper,¹² the steric effect of R is rather important.

The effects of the substituents R' are rather complicated. All of the primary and secondary aromatic amides examined show nearly the same $\Delta \nu$ values for the D₂O binary solvents. That is to say, the overall degree of hydration with D₂O is not as markedly influenced by the substituent R' in aromatic amides. On the

⁽²¹⁾ For NMCIBA, NMMeOBA, NEBA, and NPhAA, the mole fraction of water was limited to 0 to 0.8 even in D_2O solution, owing to their poor solubility in water.

⁽²²⁾ Hammett, L. P. In *Physical Organic Chemistry*; McGraw-Hill: New York, 1940; Chapter 7.



Figure 3. The amide-I band of NMClBA, NMMeOBA, NEBA, and NPhAA in dioxane-water mixtures at various mole fractions of water (X_w): (a) 0, (b) 0.073, (c) 0.11, (d) 0.14, (e) 0.24, (f) 0.32, (g) 0.41, (h) 0.54, (i) 0.80.²¹ (D₂O) N-deuteriated amides in dioxane-D₂O; (H₂O) normal species of amides in dioxane-H₂O.

contrary, the degree of hydration with H_2O is substantially influenced by the substituent R'. The $\Delta\nu$ values of the secondary benzamides with the hydrophobic R' group, namely N-alkylbenzamides such as NMBA, NMCIBA, NMMeOBA, and NEBA, are larger for H_2O than for D_2O binary solvents. However, a reverse tendency was found for the primary aromatic amides, such as benzamide and p-methoxybenzamide. Thus, an opposite H/D isotope effect was found between the secondary and primary benzamides, as given in the fourth column of Table II. Figure 1 shows that the $\Delta\nu(I-II)$ values of NMBA-d and of

NMBA are 18.5 and 23.5 cm⁻¹, respectively. The 5-cm⁻¹ larger $\Delta\nu$ (I-II) value for NMBA implies stronger C=O--HOH bonding than C=O--DOD bonding. The isotope effect of overall hydration for the secondary benzamides must be due to stronger hydration with H₂O than with D₂O at the carbonyl group. Contrary to the case of NMBA, the $\Delta\nu$ (I-II) values of benzamide-*d* and of benzamide were 17.0 and 12.0 cm⁻¹, respectively. The 5-cm⁻¹ smaller $\Delta\nu$ (I-II) value for benzamide implies weaker C=O--HOH than C=O--DOD bonding. We can summarize as follows: The strength of C=O--DOD bonding for N-alkylbenzamides-*d* is



Figure 4. Plot of $\nu_{C=0}$ vs. mole fraction of water (X_w) : D_2O and H_2O on the right of each figure indicate dioxane- D_2O and dioxane- H_2O solutions, respectively: (\blacktriangle) NMBA, (\circlearrowright) NMCIBA, (\circlearrowright) NMMeOBA, (\circlearrowright) NEBA, (\times) NPhAA.

TABL	E II:	Values	of vo	and	$\Delta \nu$
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amide	$\nu_0, {\rm cm}^{-1}$	$\Delta \nu$, cm ⁻¹	σ , ^{<i>a</i>} cm ⁻¹	$\Delta \nu_{\rm H_{2}O}/\Delta \nu_{\rm D_{2}O}$	remarks
NMBA	1668.4 ± 1.3	47.1 ± 3.9	1.4	1.32	present work
NMBA- d^b	1662.7 ± 1.0	35.8 ± 1.5	1.4		present work
NMCIBA	1672.3 ± 0.7	45.8 ± 2.2	0.9	1.35	present work
NMClBA-d	1664.1 ± 1.1	34.5 ± 2.6	1.4		present work
NMMeOBA	1664.5 ± 0.7	48.7 ± 2.4	1.0	1.42	present work
NMMeOBA-d	1657.0 ± 1.4	34.2 ± 3.2	1.7		present work
NEBA	1664.9 ± 1.0	45.3 ± 3.2	1.1	1.22	present work
NEBA-d	1659.3 ± 1.3	37.0 ± 1.3	1.0		present work
NPhAA	1697.3 ± 0.6	39.9 ± 2.1	1.0	0.96	present work
NPhAA-d	1688.5 ± 1.5	41.7 ± 3.5	1.9		present work
benzamide	1684.0 ± 0.0	25.8 ± 0.0	0.9	0.68	ref 12
benzamide-d	1668.0 ± 1.9	37.8 ± 2.4	0.1		ref 12
p-methoxybenzamide	1679.1 ± 0.0	27.4 ± 0.0	0.2	0.71	ref 12
p-methoxybenzamide-d	1664.8 ± 0.6	38.8 ± 0.9	1.6		ref 12
propionamide	1689.1 ± 2.0	31.9 ± 6.3	1.9	0.63	ref 12
propionamide-d	1676.3 ± 2.6	50.6 ± 4.2	1.6		ref 12

^aStandard deviation. ^bThe binary solvents for N-deuteriated amides are dioxane-D₂O mixtures.

almost equal to that for benzamides-d. The strength of C=O···HOH bonding is stronger than C=O···DOD bonding for N-alkylbenzamides, and vice versa for benzamides, resulting in the opposite isotope effect between primary and secondary benzamides.

NPhAA, a secondary aliphatic amide, shows little H/D isotope effect. The frequency of the amide I band of NPhAA-d and of NPhAA is 1690.5 and 1697 cm⁻¹, and that of the hydrated species II of NPhAA-d and NPhAA are 1666 and 1673 cm⁻¹, respectively. Hence the value of $\Delta \nu$ (I-II) for NPhAA-d (23.5 cm⁻¹) is nearly equal to that for NPhAA (24 cm⁻¹). The phenyl group is less hydrophobic than the alkyl group, and an amide hydrogen is even hydrophilic. The hydrophobicity of the substituent R' seems to hold the key to solving why such a dramatic difference in the H/D isotope effect was observed between primary and secondary benzamides. The isotope effect on the change of water structure by solutes with hydrophobic substituents may be responsible.

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Registry No. NMBA, 613-93-4; NMC1BA, 6873-44-5; NMMeOBA, 3400-22-4; NEBA, 614-17-5; NPhAA, 103-84-4; dioxane, 123-91-1.