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Optimal Environment for Maximal Molecular Recognition against Temperature. Three Distinct Patterns in Temperature–Recognition Profiles as a Function of Composition of Aqueous Binary Solvents

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Oxidation of a pair of associating thiols **1** and **2**, each having a binding site [–C(=O)NHC(=O)NH–] and a recognition site (R^1 or R^2), is examined at various temperatures as a function of mole fraction of water (x_w) in aqueous binary solvents with EtOH, CH_3CN, and *i*-PrOH. The selectivity (r) — a measure of the degree of molecular recognition in the oxidation — is represented by the logarithmic ratio of the yield of the unsymmetrical disulfide **4** to twice that of the symmetrical one **3**. It is found that three distinct patterns are present in temperature–recognition profiles, depending on composition of the aqueous binary solvents: (1) "progressive decrease" in the lower x_w region; (2) "presence of a maximum" in the medium x_w region; (3) "progressive increase" in the higher x_w region. It is also clarified that aqueous mixed solvents in the medium x_w region are indispensable for maximal recognition to occur against temperature (the presence of a maximum in the temperature–selectivity relationship). Dependence of the r on the x_w at lower and higher temperatures, presence of three distinct patterns in the temperature–recognition profiles, and so on are discussed in relation to the maximal recognition.

Aqueous solutions[1] have unusual physicochemical properties: (i) unique heat-capacity behavior,[2] (ii) viscosity–composition maxima[1a,3] for aqueous alcohols, (iii) striking dependence of excess enthalpies of mixing[1a] on solvent composition for aqueous alcohols, and (iv) a large negative entropy of solution[4] into water of nonpolar substances such as hydrocarbons. Considering these unique properties of aqueous media, the media might as well affect molecular recognition.

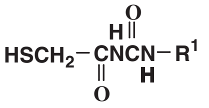
Precise and specific molecular recognition is the basis of living systems.[5] Discrimination between a large number of competing molecules of very similar structure by a given molecule can practically be accomplished by discrimination between groups involved therein.

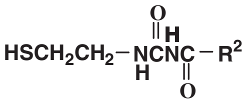
One approach to examine factors controlling precise and specific molecular recognition is to utilize model compounds in which the *essence* required for enzymes to achieve their functions (e.g., molecular recognition) is implemented. Our model compound[6,7] comprises a pair of acylurea derivatives **1** and **2**, open-chain analogues of pyrimidine bases (e.g., uracil and thymine). Thiols **1** and **2** each have three sites: (i) the reaction site (SH group) where a model reaction takes place, (ii) the binding site [–C(=O)NHC(=O)NH–, acylurea bond] whose inner NHC(=O) unit participates in two NH···O intermolecular hydrogen bonds[8,9] and which extends in the opposite direction to each other, and (iii) the recognition site (R^1 or R^2) that participates in the discrimination through specific weak intermolecular interactions.[10] Thiol **1** has the same group as a cysteine side chain ($HSCH_2$), and **2** is a derivative of cysteamine (the decarboxylated compound of cysteine).

With this model system, we have proposed the "similarity recognition" hypothesis[11] and the "shape-specific weak interaction recognition" hypothesis.[12]

In the first step of studies on the effect of aqueous media on molecular recognition, the degree of recognition of nonpolar groups was mainly examined in aqueous binary solvents, in each of which a particular mole fraction of water (x_w) was used, depending on a coexisting organic solvent [e.g., $x_w = 0.75$ (EtOH) or 0.42 (MeCN)]. As a result, it has been suggested that water in aqueous media is significant for precise and specific molecular recognition.[7]

In this study, in order to elucidate the environment optimal for *maximal molecular recognition* [i.e., the presence of a maximum in the temperature–selectivity relationship], systematic studies were further made to investigate the effect of solvent composition (mole fraction of water = x_w) on temperature dependence of molecular recognition in aqueous binary solvents. Here we report the first example demonstrating that it is in the "medium" x_w region in aqueous binary solvents, but not in pure water, that maximal recognition occurs against


$HSCH_2-\underset{\underset{O}{\|}}{C}\overset{H}{N}\overset{\overset{O}{\|}}{C}\underset{H}{N}-R^1$


$HSCH_2CH_2-\underset{H}{N}\overset{\overset{O}{\|}}{C}\overset{H}{N}\underset{\underset{O}{\|}}{C}-R^2$

1a $R^1 = C_5H_{11}$ **2a** $R^2 = C_5H_{11}$

1b $R^1 = C_6H_5$ **2b** $R^2 = C_4H_9$

Chart 1.

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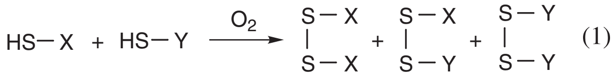
temperature.

Oxidation[13] [Eq. 1] of a pair of thiols **1** and **2** (Chart 1) was examined as a model reaction for discrimination. The selectivity (r) in the oxidation — a measure of the degree of molecular recognition, more exactly, recognition of **1** by **2** (or of **2** by **1**) — is defined as the logarithmic ratio of the yield of the unsymmetrical disulfide **4** to twice that of the symmetrical disulfide **3**:

$$r = \ln\{[\mathbf{4}]/2[\mathbf{3}]\}.$$



$$\mathrm{HS{-}X} + \mathrm{HS{-}Y} \xrightarrow{O_2} \begin{matrix}\mathrm{S{-}X}\\ |\\ \mathrm{S{-}X}\end{matrix} + \begin{matrix}\mathrm{S{-}X}\\ |\\ \mathrm{S{-}Y}\end{matrix} + \begin{matrix}\mathrm{S{-}Y}\\ |\\ \mathrm{S{-}Y}\end{matrix} \quad (1)$$

1 **2** **3** **4** **5**

Therefore, when the three disulfides **3**, **4**, and **5** are formed in the statistical ratio (i.e., 1:2:1 ratio), the r becomes zero.

Results

Temperature Dependence of Molecular Recognition in Aqueous Binary Solvents. Figure 1 shows temperature dependence of the selectivity (r) for $R^1 = R^2 = C_5H_{11}$ as a function of mole fraction of water (x_w) in H_2O–EtOH in lower (A), medium (B), and higher x_w regions (C). When the x_ws are in the lower region (Fig. 1A), the r has all been found to decrease progressively with increasing temperature. This decreasing pattern is generally observed for selectivity in ordinary organic reactions.

In the medium x_w region (Fig. 1B), the r has all proved to exhibit a maximum at 35 °C, the maximum being sharpest for $x_w = 0.75$. These results (Figs. 1A and 1B) demonstrate that pattern of the temperature dependence of the r alters from progressive decrease to a bell-shaped curve, by very slight changes in the x_w of 0.02 (i.e., from 0.48 to 0.50).

In the higher x_w region (Fig. 1C), the r has all been shown to increase progressively with increasing temperature. Pattern of the temperature dependence varies from a bell-shaped curve to progressive increase also by very slight changes in the x_w of 0.02 (i.e., from 0.80 to 0.82 in Figs. 1B and 1C).

Figure 2 shows temperature dependence of the selectivity (r) for $R^1 = R^2 = C_5H_{11}$ as a function of the x_w in H_2O–MeCN in lower (A), medium (B), and higher (C) x_w regions. When the x_ws are in the lower region (Fig. 2A), the r has all been found to decrease progressively with increasing temperature. At the lowest x_w (0.10) examined, the temperature dependence is smallest.

In the medium x_w region (Fig. 2B), the r has all turned out to exhibit a maximum at about 35 °C; the maximum is sharpest for $x_w = 0.42$, the value corresponding to 80% MeCN (v/v) in H_2O–MeCN.

In the higher x_w region (Fig. 2C) ($x_w = 0.75$ and 0.90), the r has all been shown (i) to increase progressively with temperature in the 20–50 °C range and (ii) to slightly decrease with temperature in going from 50 to 70 °C. Therefore, in this region, the r each exhibits a vague maximum at about 50 °C.

Figure 3 shows the temperature dependence of the r for $R^1 = R^2 = C_5H_{11}$ as a function of the x_w in H_2O–i-PrOH. Also in this case, in the lower x_w region (0.20), the r decreases with temperature; in the medium x_w region (0.50, 0.75, and 0.80), the r each exhibits a maximum (at 35 °C); in the higher

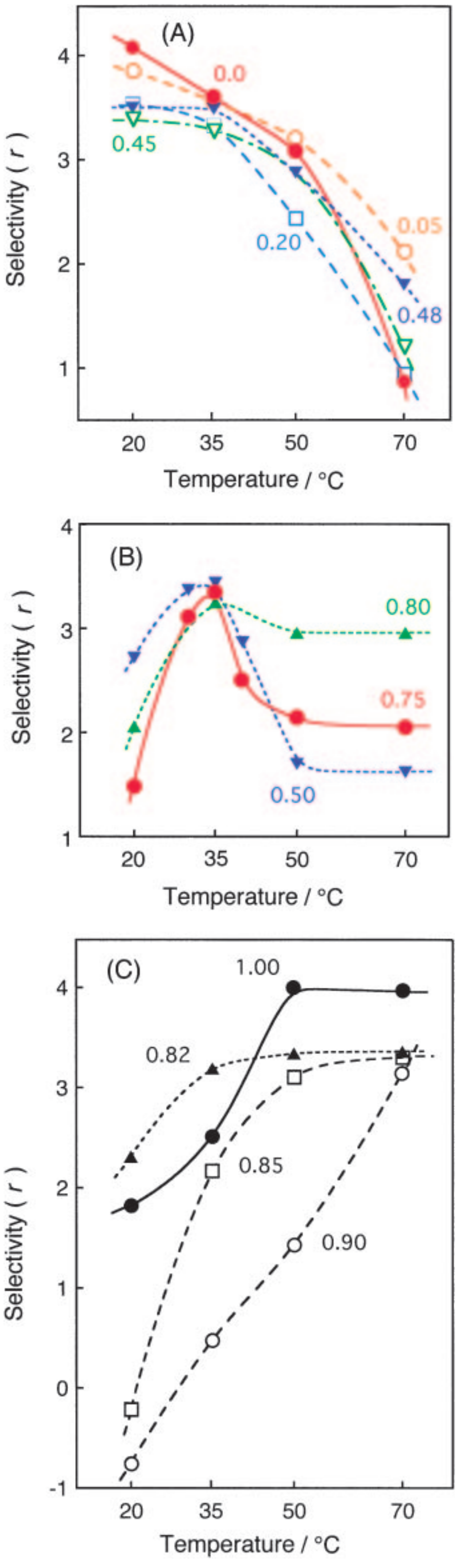


Fig. 1. Temperature dependence of the selectivity (r) for $R^1 = R^2 = C_5H_{11}$ as a function of mole fraction of water (x_w) in H_2O–EtOH in lower (A), medium (B), and higher x_w regions (C). The numerals in this figure are the x_w. Errors [three times the standard deviations (3σ)] for r values range from ±0.01 to ±0.24, except at $x_w = 0.85$ at 20.0 °C (±0.32) and $x_w = 1.00$ at 50.0 °C (0.43), about half of which range from ±0.01 to ±0.09.

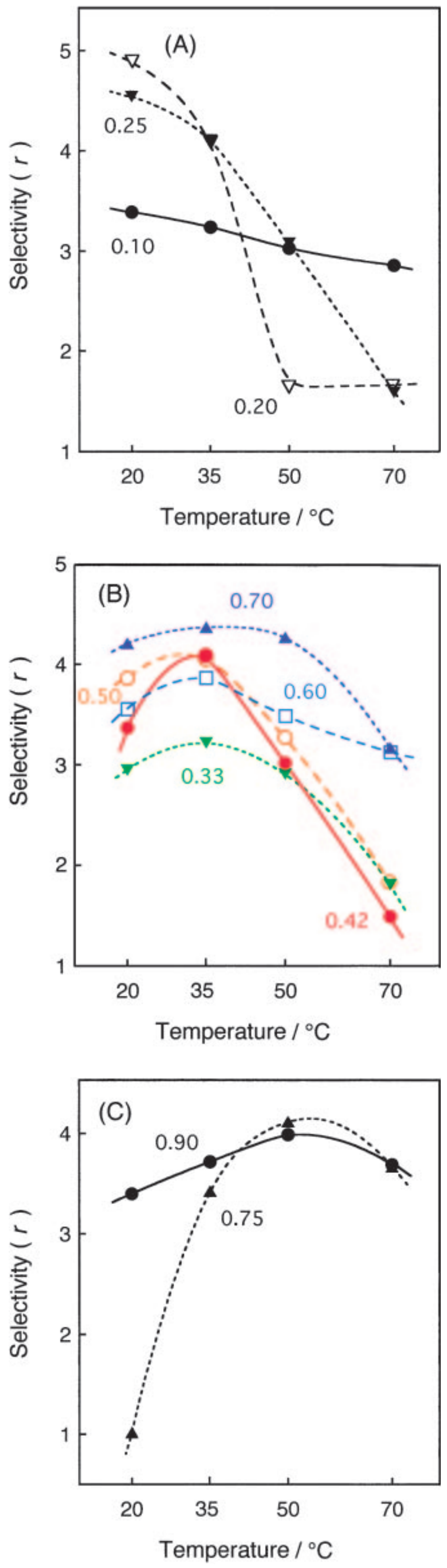


Fig. 2. Temperature dependence of the selectivity (r) for $R^1 = R^2 = C_5H_{11}$ as a function of the x_w in H_2O–MeCN in lower (A), medium (B), and higher x_w regions (C). The numerals in this figure are as in Fig. 1. Errors (3σ) for r values range from ±0.01 to ±0.22 except at $x_w = 0.25$ at 70 °C (±0.26).

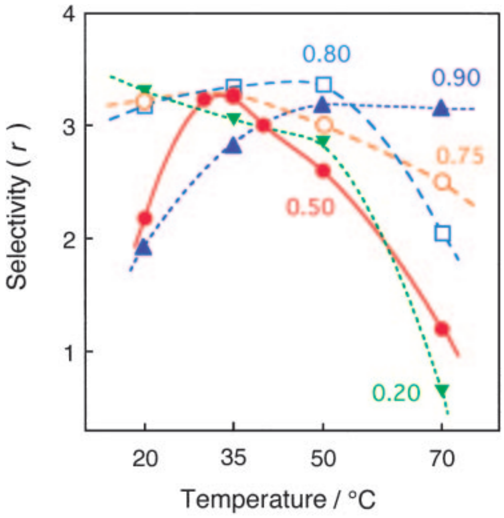


Fig. 3. Temperature dependence of the selectivity (r) for $R^1 = R^2 = C_5H_{11}$ as a function of the x_w in H_2O–i-PrOH. The numerals in this figure are as in Fig. 1. Errors (3σ) for r values range from ±0.02 to ±0.26 except at $x_w = 0.50$ at 20.0 °C (±0.32) and at $x_w = 0.80$ at 50.0 °C (±0.34).

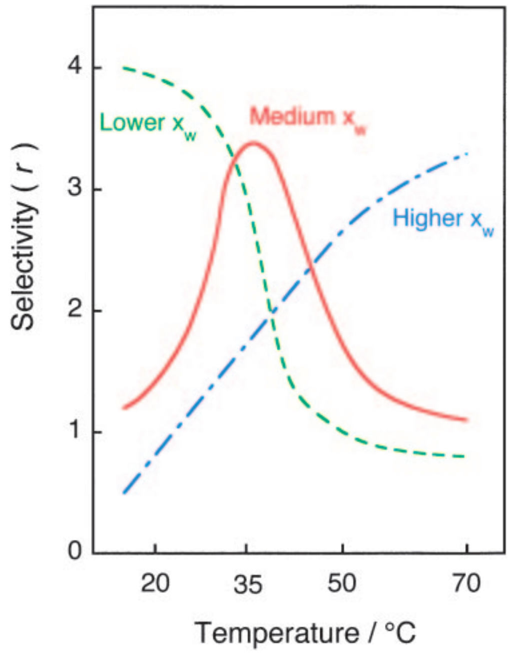


Fig. 4. Schematic drawing of temperature dependence of the selectivity (r) as a function of the x_w in aqueous binary solvents.

x_w region (0.90), the r increases with temperature.

Figure 4 shows a schematic drawing of the *three distinct patterns* in the temperature–recognition profiles as a function of the x_w in aqueous binary solvents, based on the data shown in Figs. 1–3. (1) In the lower x_w region, the r decreases progressively with increasing temperature. (2) In the medium x_w region, the r exhibits a maximum, very sharp at least at a particular x_w (e.g., 0.75, 0.42, or 0.50). (3) In the higher x_w region, the r increases progressively with temperature.

Temperature Dependence of Molecular Recognition in

Nonaqueous Binary Solvents. Figure 5 illustrates temperature dependence of the selectivity (r) for $R^1 = R^2 = C_5H_{11}$ in nonaqueous binary solvents containing EtOH ($x_{EtOH} = 0.25$). When the other component is not water but MeOH or 1,4-dioxane ($x = 0.75$), the r simply decreases with temperature.

The experimental evidence shown in Figs. 1–3 and 5 demonstrates that aqueous mixed solvents having the medium x_ws are indispensable for *maximal recognition* to occur against temperature.

Influence of Structures of Recognition Sites on Temperature Dependence of Molecular Recognition. Figure 6 illustrates temperature dependence of the selectivity (r) for R^1 = Ph and $R^2 = C_4H_9$ as a function of the x_w in H_2O–EtOH. Also for this combination of R^1 and R^2, the three distinct patterns are observed. When the x_w is 0.10 and 0.50, the r decreases progressively with temperature. For $x_w = 0.75$, the r shows a broad maximum at about 35 °C; for $x_w = 0.90$, the r increases with temperature.

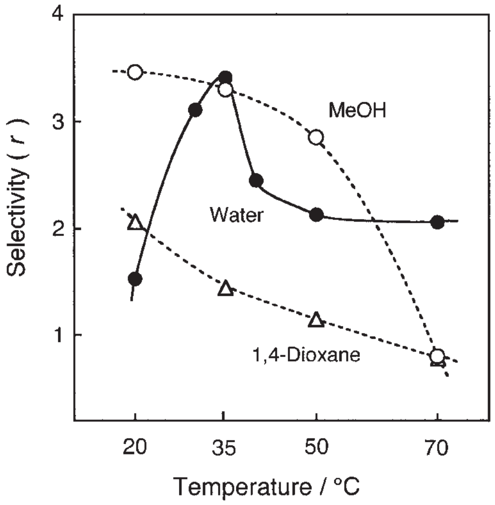


Fig. 5. Temperature dependence of the selectivity (r) for $R^1 = R^2 = C_5H_{11}$ as a function of a coexisting organic solvent mixed with EtOH ($x_{EtOH} = 0.25$). The coexisting organic solvents are shown in this figure. Errors (3σ) for r values range from ±0.06 to ±0.27.

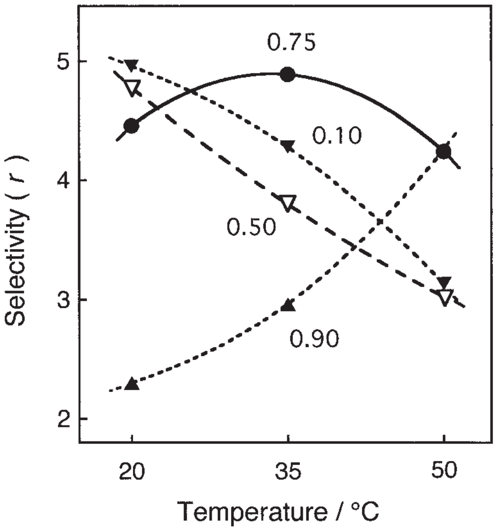


Fig. 6. Temperature dependence of the selectivity (r) for R^1 = Ph and $R^2 = C_4H_9$ as a function of the x_w in H_2O–EtOH. The numerals in this figure are as in Fig. 1. Errors (3σ) for r values range from ±0.03 to ±0.28, except at $x_w = 0.10$ at 50.0 °C (±0.30) and at $x_w = 0.90$ at 20.0 °C (±0.34).

Figure 7 displays temperature dependence of the selectivity (r) for R^1 = Ph and $R^2 = C_5H_{11}$ as a function of the x_w in H_2O–EtOH. In the case of this combination of R^1 and R^2, the r has all been found to increase progressively with temperature, regardless of the x_w employed (0.10, 0.50, 0.75, and 0.90). The experimental results shown in Figs. 1–3, 6, and 7 are in accord with our hypothesis[12] that specific weak interactions between R^1 and R^2 would give decisive effect on molecular recognition in aqueous binary solvents.

Discussion

Mechanism of the Oxidation.[7] It has been clarified that the product ratio in this type of oxidation is kinetically controlled on the following basis: (1) the product ratios do not change as the oxidation proceeds; (2) a thiol–disulfide exchange reaction takes place only slowly under conditions similar to those for the oxidation.[6,14] The formation of dimers consisting of **1** and **2**, suggested by ^{1}H NMR and IR spectroscopic studies, cannot explain the r values in some cases.[7,12] The tetramers

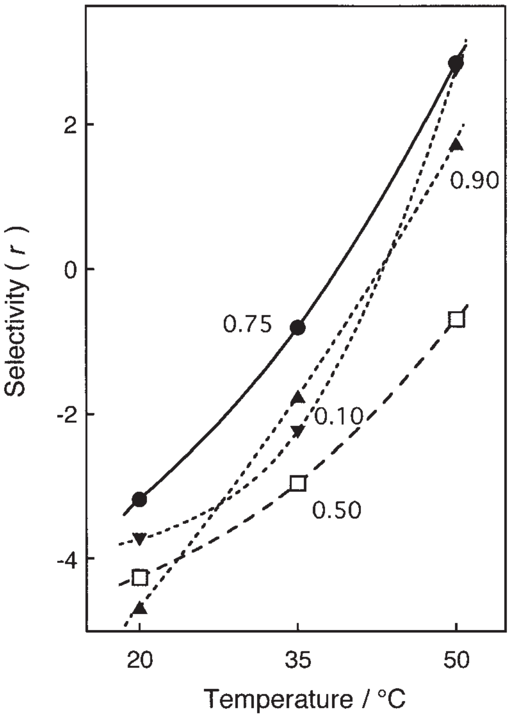


Fig. 7. Temperature dependence of the selectivity (r) for R^1 = Ph and $R^2 = C_5H_{11}$ as a function of the x_w in H_2O–EtOH. The numerals in this figure are as in Fig. 1. Errors (3σ) for r values range from ±0.06 to ±0.28. The r values for $x_w = 0.75$ at 35.0 °C (−0.80) represent the mean values for ten experiments, because r values ranged largely from 2.97 to −4.04. The reason remains unknown.

(two homotetramers and three heterotetramers) formed by dimerization of the dimers have been suggested to be intermediates in this oxidation.[8,15]

Intermolecular Association. Intermolecular association has been demonstrated to be the first requirement for molecular recognition.[6] This view is consistent with the present work in that, at higher temperatures (conditions unfavorable for intermolecular hydrogen bonds), the r in the lower x_w region approaches zero (the value statistically expected) (Figs. 1A and 3).

The decrements in the r with increasing temperature, the phenomena usually observed for selectivity in ordinary organic reactions, would be explained by weakening of the intermolecular association between associating thiols **1** and **2** with increasing temperature.[16]

Dependence of the r on the x_w at Lower and Higher Temperatures. Examination of the dependence of the r on the x_w at lower temperatures (e.g., 20 °C) (Figs. 1–3) reveals that the r values tend to be reduced, as a whole, in increasing order of x_ws (lower > medium > higher x_w regions, Figs. 1 and 3). This finding may be due to weakening of the intermolecular association with increasing x_ws.

On the other hand, the r values at higher temperatures tend to increase, as a whole, in increasing order of the x_w in the three distinct regions (lower < medium < higher x_w regions, Figs. 1B, 2B, and 3).

Hydrophobicity (the strength of hydrophobic interaction[17]) is reported to increase progressively with increasing temperature.[4] Moreover, hydrophobicity is shown to increase progressively with the x_w in aqueous EtOH ($x_w \leq$ ca. 0.85 at 30 °C).[18]

At higher temperatures, the dependence of the r on the x_w regions mentioned above might agree roughly with the dependence of hydrophobicity on the x_w. Thus, hydrophobic interaction would be one factor controlling the r at higher temperatures.

Presence of Three Distinct Patterns in Temperature–Discrimination Profiles. One might expect that progressive changes in the r with the x_w would occur in aqueous mixed solvents for the temperature–discrimination profiles. As is evident from the above results, however, the temperature–discrimination profiles can be classified into the three distinct patterns (i.e., progressive decrease, a bell-shaped curve, and progressive increase) corresponding to the lower, medium, and higher x_w regions, respectively.

A possible interpretation is that the observed changes in the pattern would be related to changes in solvent structures, probably due to changes in hydrogen bonds among water and organic solvent molecules, weak interactions among alkyl groups in aqueous organic solvents, and so on, at the two critical x_ws [i.e., (1) about 0.50 and (2) about 0.8 (Fig. 1)]. In this connection, by measuring the enthalpies of transfer of tetraalkylammonium halides in *i*-PrOH–water mixtures, the change in solvent structure has been suggested to occur over a relatively small range of solvent compositions at around 0.25 volume fraction of the alcohol.[19] Further, it is interesting to note that in aqueous solutions there are two mole-fraction regions (x_w = ca. 0.2 and 0.8) in which bulk properties of aqueous solutions undergo dramatic changes.[1b]

Optimal Environment for Maximal Recognition to Occur against Temperature. The most fascinating aspect of the present work is that it is aqueous mixed solvents having the medium x_ws that are required for the presence of a maximum in the temperature–selectivity profiles (i.e., *maximal recognition*) (Fig. 4). It is rather astonishing that the x_ws optimal for maximal recognition are not higher.

Maximal recognition is considered to result from two opposing physical properties against temperature in aqueous mixed solvents, the x_ws of which are in the medium range.

Our view concerning the two possible factors having opposing physical properties against temperature is as follows. (1) One would be weakening of intermolecular association through intermolecular hydrogen bonds, dominant in the higher temperature range (from the temperature at which maximal discrimination occurs to 70 °C), thus causing the decrement of r with temperature. (2) The other factor, dominant in the lower temperature range, thereby causing the increment in the r with temperature, remains to be elucidated. A factor to be examined would be changes in solvent structures, which would affect the intermolecular association, thus resulting in changes in the selectivity.

Further studies will be required for the elucidation of the reason for maximal recognition against temperature.

Conclusion

By using a pair of model compounds each participating in (i) two intermolecular hydrogen bondings and (ii) shape-specific weak interactions, it has been demonstrated that there exist the *three distinct patterns* in temperature–recognition profiles as a function of composition of aqueous binary solvents. (1) In the lower x_w region, the degree of recognition decreases progressively with increasing temperature. (2) In the medium x_w region, the degree of recognition exhibits a maximum, very sharp at least at a particular x_w (e.g., 0.75, 0.42, or 0.50). (3) In the higher x_w region, the degree of recognition increases progressively with temperature.

The present work suggests that aqueous binary solvents having the medium x_ws are significant for the *maximal recognition* to occur against temperature.

Experimental

General Procedures. ^{1}H NMR spectra were recorded with a JEOL GX-270 spectrometer. Chemical shifts (δ) are reported downfield from internal $SiMe_4$. Low- and high-resolution mass spectra were obtained on a JEOL JMS-DX303 mass spectrometer. Melting points were determined on a Yamato oil-immersion apparatus and are uncorrected. HPLC separations were conducted on a Waters system (515 pump, 486 UV detector, and Millennium 32).

Materials. The alcohols and acetonitrile used were all of HPLC grade (Wako, Tokyo) and were used as such. Water was purified through Millipore Milli-Q water purification system followed by distillation.

Preparation of Thiols.[7] Thiols **1** were prepared by reaction of the corresponding *S*-esters [$MeC(=O)SCH_2C(=O)NHC(=O)NH–R^1$] with cysteamine as described previously.[7] Their properties were reported previously.[7,20] Thiols **2** were prepared by addition of the corresponding acyl isocyanates to freshly sublimed cysteamine in tetrahydrofuran (THF) under argon at 0 °C as described previously.[14] Their properties were reported previously.[21]

Preparation of Disulfides.[14] Symmetrical disulfides **3** were easily obtained by treatment of **1** with O_2 in the presence of Et_3N in MeCN at room temperature, and recrystallized from THF–dichloromethane. Unsymmetrical disulfides **4** were prepared by repeated recrystallization of the corresponding oxidation mixtures. The properties of disulfides **3** and **4** were reported previously.[7,22,23]

Oxidation of a Pair of Thiols. A mixture of **1** (0.50 mmol) and **2** (0.50 mmol) in a solvent (12.5 cm^3) was stirred vigorously under oxygen for 15 min in a well-stirred water bath which was thermostated to ±0.1 °C for 20 to 50 °C and to ±0.5 °C for 70 °C. To this mixture was added Et_3N (0.05 mmol), and vigorous stirring was continued for the time required to complete the oxidation (the oxidation was performed at least twice under the same conditions). When the oxidation was completed, the reaction mixture was evaporated to dryness. The yields of **3** and **4** were determined by using their absorption at 251 nm after separation of the three disulfides in the mixture by HPLC using LiChrosorb CN with hexane-*i*-PrOH (98.5:1.5) as an eluent. The *r* values given in Figs. 1–3 and 5–7 represent the mean values for two or more experiments, and were reproducible within the errors shown therein.

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