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Aqueous Media Effect on Molecular Recognition. II.[1)] Temperature Dependence of Chemical Selectivity in Alcohols and Water

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Oxidation of a pair of associating thiols (**1** and **2**), each having the binding site [–C(=O)NHC(=O)NH–] and the recognition site (R^1 or R^2), is examined at several temperatures in alcohols (MeOH, EtOH, *n*-PrOH, and *i*-PrOH) and water. The selectivity (r), a measure of molecular recognition of **1** by **2** (or of **2** by **1**), in the oxidation is defined as the logarithmic ratio of the yield of an unsymmetrical disulfide (**4**) to twice that of a symmetrical one (**3**). It is found that the selectivity in the alcohols each decreases markedly with increasing temperature except for one case, whereas that in water increases with increasing temperature. Correlation of the observed selectivity with factors affecting the selectivity (e.g., intermolecular association, physicochemical properties of solvents, and hydrophobic interaction) is discussed.

Aqueous solutions[2)] have unique physicochemical properties. These include (i) unique heat capacity behavior of aqueous solutions,[3)] (ii) viscosity–composition maxima[2a,4)] for aqueous alcohols, (iii) striking dependence of excess enthalpies of mixing[2a)] on solvent composition for aqueous alcohols, and (iv) a large negative entropy of solution[5)] into water of nonpolar substances such as hydrocarbons.

Highly specific enzymatic reactions occur in aqueous media. *Nonenzymatic* reactions might also be expected to proceed specifically in *aqueous* media, if conducted in ordered molecular aggregates where specific weak interactions operate.

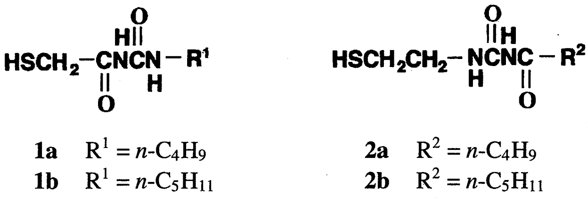
Molecular recognition is vital to living systems. In order to elucidate factors controlling such precise molecular recognition as occurs in living systems, we have been studying the selectivity (a measure of molecular recognition) in oxidation of a pair of associating thiols in aqueous and the corresponding nonaqueous media. As a result, it has been demonstrated that (i) three-dimensional shape similarity between interacting groups in reacting molecules is responsible for more specific and precise molecular recognition than would otherwise be achieved[6)] and (ii) degree of the recognition between molecules each having a nonpolar group can be controlled by the strength of shape-specific weak interactions between nonpolar groups in the respective molecules.[7)]

Recently we have suggested that *aqueous mixed solvents* would *amplify* the degree of *recognition* of nonpolar groups in narrow environment (e.g., solvent composition and temperature) range in molecular aggregates.[1,8)] As a continuing study on this subject, investigations have been made on whether or not the amplification of the recognition occurs in a pure organic solvent and in pure water. This paper describes temperature dependence of the recognition of nonpolar groups in some alcohols and water together with that in aqueous alcohols for reference.

Our model compound consists of a pair of acylurea derivatives (**1** and **2**), open-chain analogs of pyrimidine bases (Chart 1). 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[6b,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. In this study, we used six reaction systems: systems A, B, C, D_4, D_{i5}, and D_6; among these, systems A and D_{i5} were used in the preceding paper.[1)] System A is composed of a 1 : 1 mixture of **1a** and **2a**, B of **1b** and **2b**, and so on (Chart 2).

As a model reaction, oxidation with oxygen was chosen, in view of the finding that the correct pairing of half-cystine residues in proteins (i.e., specific S–S bond formation) has been suggested to depend upon specific noncovalent bonds.[10)]

Oxidation of a 1 : 1 mixture of **1** and **2** with oxygen in the presence of a catalytic amount of triethylamine gives one



$$\mathrm{HSCH_2{-}\underset{\underset{\displaystyle O}{\|}}{C}\overset{H}{N}\overset{\overset{\displaystyle O}{\|}}{C}\underset{H}{N}{-}R^1} \qquad \mathrm{HSCH_2CH_2{-}\underset{H}{N}\overset{\overset{\displaystyle O}{\|}}{C}\overset{H}{N}\underset{\underset{\displaystyle O}{\|}}{C}{-}R^2}$$

1a $R^1 = n\text{-}C_4H_9$
1b $R^1 = n\text{-}C_5H_{11}$
1c $R^1 = n\text{-}C_{10}H_{21}$
1d $R^1 = Ph$

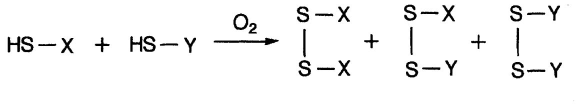
2a $R^2 = n\text{-}C_4H_9$
2b $R^2 = n\text{-}C_5H_{11}$
2c $R^2 = n\text{-}C_{10}H_{21}$
2d $R^2 = i\text{-}C_5H_{11}$
2e $R^2 = n\text{-}C_6H_{13}$

Chart 1.

System A : **1a** + **2a**
System B : **1b** + **2b**
System C : **1c** + **2c**
System D_4 : **1d** + **2a**
System D_{i5} : **1d** + **2d**
System D_6 : **1d** + **2e**

Chart 2.

unsymmetrical (**4**) and two symmetrical disulfides (**3** and **5**) (Eq. 1).



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

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

The selectivity (r)—a measure of the degree of the recognition of **1** by **2** (or of **2** by **1**) — is defined as the logarithmic ratio of the yield of unsymmetrical disulfide **4** to twice that of symmetrical disulfide **3** (Eq. 2).

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

Therefore, when the three disulfides (**3**, **4**, and **5**) are formed in the statistical ratio (i.e., 1 : 2 : 1 ratio), the r becomes 0.

Results and Discussion

Temperature Dependence of the Selectivity in Alcohols. The selectivity was first examined in some alcohols, with temperature being changed.

Figure 1 shows temperature dependence of the selectivity (r) for systems A, B and C in EtOH. The r for each system has turned out to decrease largely with increasing temperature, regardless of the reaction systems used.

Figure 2 plots the r for system D_{i5} against temperature in MeOH, EtOH, *n*-PrOH, and *i*-PrOH. The r in these alcohols has also proved to decrease markedly with temperature. It is interesting to note that the temperature–selectivity curves in MeOH and EtOH show symmetry as a whole.

In Fig. 3 is given temperature dependence of the selectivity for system A in MeOH and EtOH. The r in the two alcohols each decreases with temperature. There is considerable difference in pattern of the temperature dependence of the r in EtOH between systems A (Fig. 3) and D_{i5} (Fig. 2).

Figure 4 illustrates a plot of the selectivity in EtOH for systems D_4 and D_6 against temperature. The r for system D_4 is also reduced with increasing temperature, whereas that for system D_6 increases from −4.0 (20 °C) to −1.4 (50 °C) with increasing temperature.

These results demonstrate that, in *alcohols*, the selectivity *decreases* (or increases in one case) markedly with increasing temperature.[11]

Temperature Dependence of the Selectivity in Water. The selectivity in pure *water*[12] was then investigated as a function of temperature for systems B, C, and D_{i5}. As is evident from the data in Fig. 5, the selectivity in water *increases* with increasing temperature.

A fascinating aspect of the data (Fig. 5) is that the r in water is much higher for system B (R^1=R^2=n-C_5H_{11}) than for system C (R^1=R^2=n-$C_{10}H_{21}$) regardless of temperature, as is the case with that in EtOH (Fig. 1).[13]

Temperature Dependence of the Selectivity in Aqueous Alcohols. In connection with the amplification of the

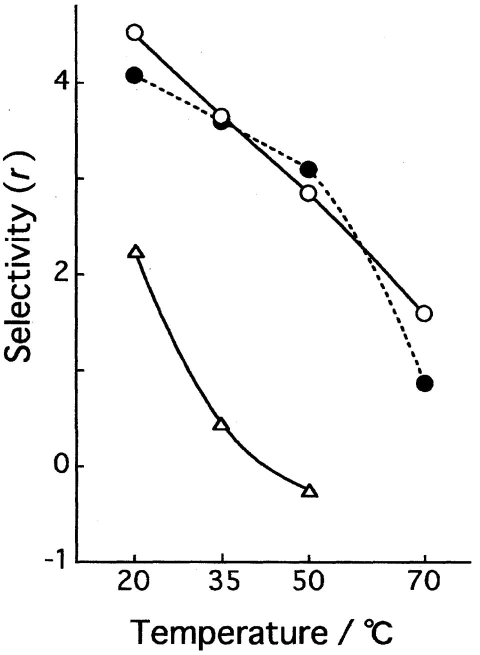


Fig. 1. Temperature dependence of the selectivity (r) in EtOH for systems A (○), B (●), and C (△). Errors (three times the standard deviations) for r values range from ±0.07 to ±0.17 except for system B at 35 °C (±0.28), 50 °C (±0.24), and 70 °C (±0.30).

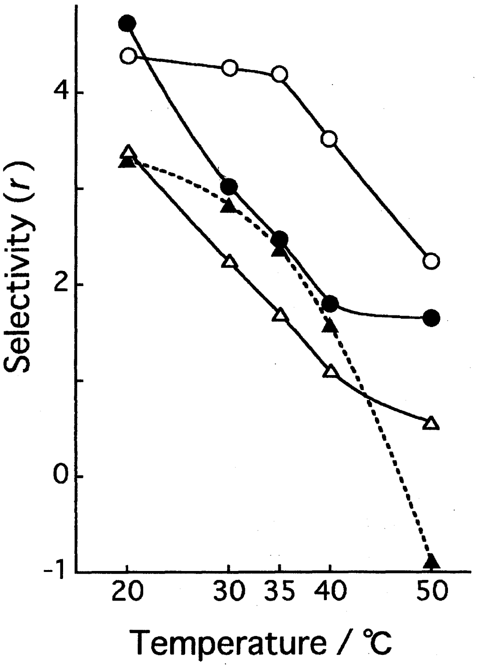


Fig. 2. Temperature dependence of the selectivity (r) for system D_{i5} in MeOH (○), EtOH (●), *n*-PrOH (△), and *i*-PrOH (▲). Errors (three times the standard deviations) for r values range from ±0.01 to ±0.12 except in *n*-PrOH at 30 °C (±0.21).

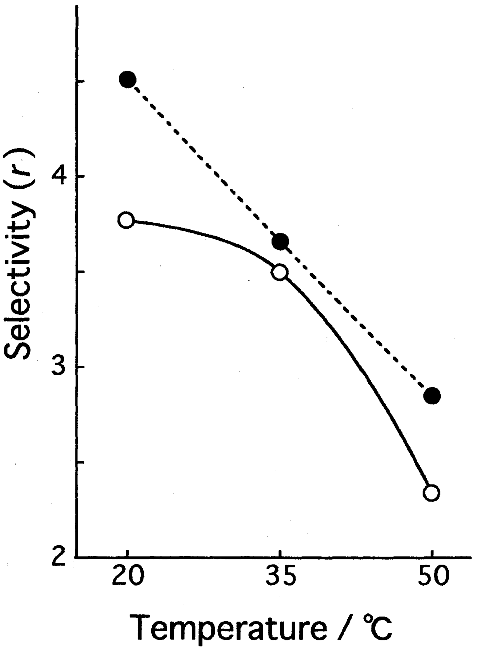


Fig. 3. Temperature dependence of the selectivity (r) for system A in MeOH (○) and EtOH (●). Errors (three times the standard deviations) for r values range from ±0.03 to ±0.10 except in MeOH at 20 °C (±0.19).

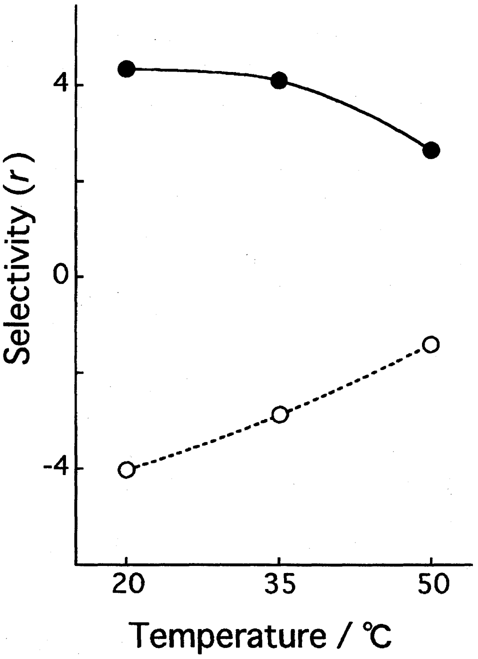


Fig. 4. Temperature dependence of the selectivity (r) in EtOH for systems D_4 (●) and D_6 (○). Errors (three times the standard deviations) for r values range from ±0.04 to ±0.16 except for system D_4 at 20 °C (±0.34) and 35 °C (±0.27).

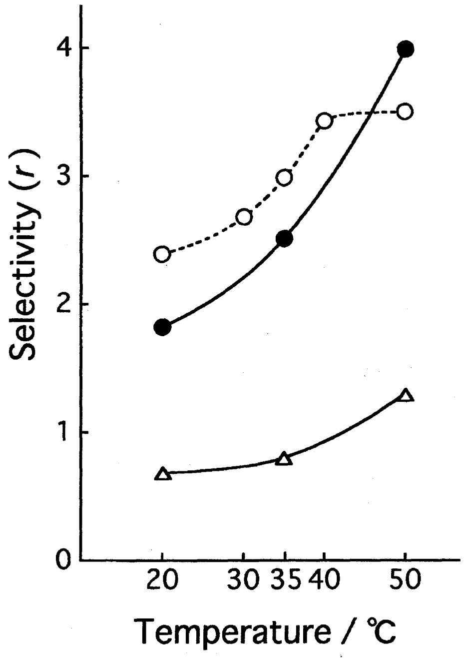


Fig. 5. Temperature dependence of the selectivity (r) in H_2O for systems B (●), C (△), and D_{i5} (○). Errors (three times the standard deviations) for r values range from ±0.12 to ±0.23 except for system B at 50 °C (±0.43).

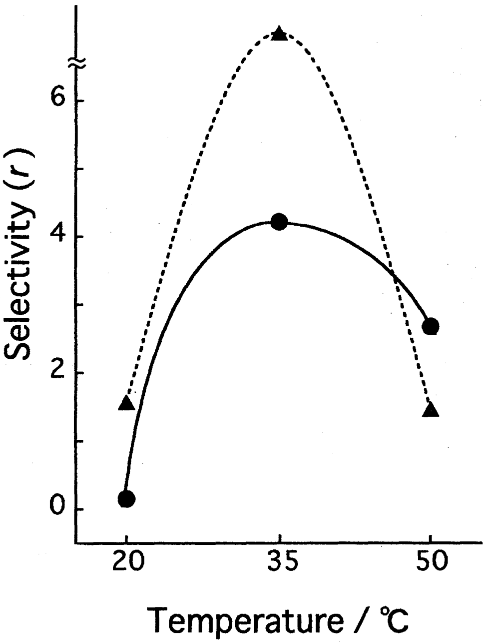


Fig. 6. Temperature dependence of the selectivity (r) for system D_{i5} in water–EtOH [mole fraction (x) of water (x_w)= 0.70] (●) and water–i-PrOH (x_w=0.80) (▲). Errors (three times the standard deviations) for r values range from ±0.04 to ±0.08.

recognition mentioned above,[1,8] temperature dependence of the selectivity in aqueous alcohols was examined for system D_{i5} (Fig. 6).

The r in water–EtOH [mole fraction (x) of water (x_w)= 0.70] and water–i-PrOH (x_w=0.80) has each been found to become very high around 35 °C. This indicates that aqueous alcohols make specific molecular recognition easy to occur in a certain temperature range.

In contrast, the selectivity decreases or increases progressively with increasing temperature in alcohols or water, respectively (Figs. 1, 2, 3, 4, and 5). Therefore, specific molecular recognition occurs only in aqueous alcohols in a certain temperature range.

Such "amplification" effect in molecular recognition is observed in aqueous mixed solvents also in the case of their solvent composition being changed.[1]

In what follows, we wish mainly to discuss factors affecting the selectivity: (i) reactivity difference between thiols **1** and **2**, (ii) intermolecular association, (iii) physicochemical properties of solvents, (iv) hydrophobic interaction, and (v) a possible explanation of changes in pattern of temperature dependence of recognition.

Mechanism of the Oxidation.[1] It has been demonstrated that the product ratio in this type of oxidation is kinetically controlled on the basis of the observations that (i) the product ratios do not change as the oxidation proceeds and (ii) a thiol-disulfide exchange reaction[14] takes place only slowly under conditions similar to those for the oxidation.[15,16]

Reactivity Difference between Thiols 1 and 2. The large reactivity difference between thiols[17] **1** and **2** is not responsible for the observed selectivity. This is because *r* values should become negative regardless of the reaction systems employed, if the selectivity depends upon the reactivity difference; however, the *r* shows positive values for all reaction systems (Figs. 1, 2, 3, 4, 5, and 6).[18]

Tetramers as Reaction Intermediates. ^{1}H NMR and IR spectroscopic studies of **1** and **2** revealed that **1** and **2** formed weak complexes in solution with each other as well as with themselves through two NH···O intermolecular hydrogen bonds.[9a] Since the two acylurea bonds in **1** and **2** extend in the opposite direction to each other, the association patterns of homodimers **6** and **8** are of head-to-tail type, that of heterodimer **7** being of head-to-head type (Fig. 7).

In homodimer **6** or **8**, the distance between the two HS groups is too long for the S–S bond to be formed. Therefore, homodimers cannot explain the *selective* formation of *symmetrical* disulfides (i.e., $r<0$) in some cases: (i) system D_{i5} in *i*-PrOH at 50 °C (Fig. 2), (ii) system D_6 (Fig. 4), and (iii) other reaction systems used previously.[6a,6c,7,15,16,17b]

X-Ray crystallographic study of thiol **1** has indicated that there exist four molecules in the unit cell ($Z=4$).[9b] Moreover, unsymmetrical disulfide **4** was shown (i) to adopt a U-shaped conformation and (ii) to form *dimers* in solution;[19] the dimers can be regarded as corresponding to *tetramers* composed of thiols **1** and **2**.

On the basis of these findings, tetramers [two homotetramers (**9** and **10**) and three heterotetramers (**11**, **12**, and **13**)] have been suggested to be intermediates in this oxidation (Fig. 8).[6b,9a,20]

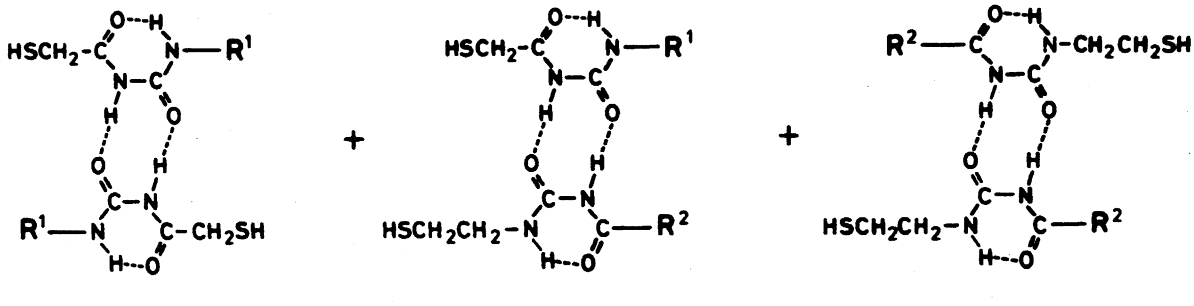
Each tetramer would afford the corresponding disulfide(s) selectively when treated with oxygen: (i) heterotetramers **11** and **12**, and probably **13**, would exclusively give unsymmetrical disulfide **4** and (ii) homotetramers **9** and **10** would exclusively give symmetrical disulfides **3** and **5**, respectively. Relative concentrations of tetramers, which are considered to control the selectivity,[17a] depend primarily upon the reaction systems employed.

Intermolecular Association. Intermolecular association has been demonstrated to be the first requirement for molecular recognition.[15,21,22] This view also receives support from the present work in that, at higher temperatures (conditions unfavorable for intermolecular association through two intermolecular hydrogen bonds[23]), the *r* approaches 0 (the value statistically expected) (i) for system B (Fig. 1) and (ii) for system D_{i5} in *n*-PrOH (Fig. 2).

The decrement in the *r* with increasing temperature — the phenomenon which is generally observed for selectivity in ordinary organic reactions — in alcohols would be explained by the weakening in intermolecular association between associating thiols (**1** and **2**) with temperature.

Physicochemical Properties of Solvents. Investigations were made as to whether or not there exists relationship between physicochemical properties of solvents and the observed selectivity.

First, dielectric constants (ε) of alcohols (MeOH, EtOH, and *n*-PrOH) and water are both reduced with increasing temperature,[24] whereas the pattern of temperature dependence of the *r* in alcohols and water is reversed for systems B, C, and D_{i5} (Figs. 1, 2, and 5). Second, the order of viscosity (η)[4b] and critical temperature (T_c)[12c] of ROH (R = H and alkyl groups), each having a minimum at R = Me, for the number of carbon atoms (*n*) of ROH does not agree with that of the *r* for the *n* (at 20 and 50 °C in Figs. 2 and 5). Third, the δ_{SA} (a measure of self-association energy)[25] of ROH (R = H and alkyl groups) is reported to decrease with increasing *n* of ROH. However, the order of the δ_{SA} for the *n* does not agree with that of the *r* for the *n* (at 20, 30, 35, and 40 °C in



6 **7** **8**

Fig. 7. Association patters of dimers **6**, **7**, and **8**. - - -, Hydrogen bonding.

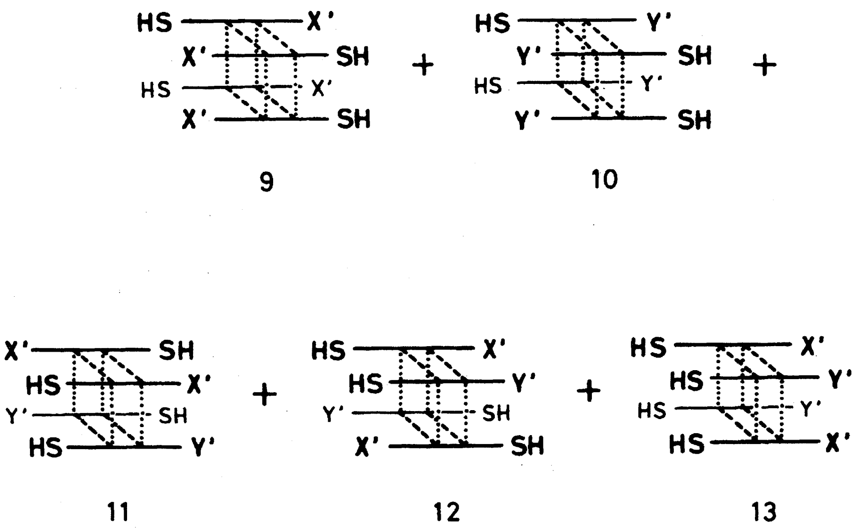


Fig. 8. Association schemes of tetramers **9**, **10**, **11**, **12**, and **13** formed by dimerization of dimers **6**, **7**, and **8**. ---, Hydrogen bonding responsible for the stabilization of dimers; ····, noncovalent weak interactions responsible for the stabilization of tetramers. Symbols X′ and Y′ represent R^1 and R^2, respectively.

Figs. 2 and 5).

Therefore, it seems reasonable to consider that the physicochemical properties of the solvents would affect the selectivity (Figs. 1, 2, and 5), but any one of them cannot explain it by itself.

Hydrophobic Interaction.[26)] As mentioned above, relative concentrations of tetramers (i) are considered to control the selectivity and (ii) would depend upon stabilities of the tetramers, each tetramer affording the corresponding (i.e., particular) disulfide(s) selectively when treated with oxygen.

In the case of systems B and C, it is almost impossible to anticipate which of the tetramers [i.e., homo- (**9** and **10**) or heterotetramers (**11**, **12** and **13**)] is more stable, since R^1 and R^2 are identical in each system. This is because it is not clear which of the interactions between recognition sites (R^1–R^1 and R^2–R^2 interactions in **9** and **10** or R^1–R^2 interactions in **11**, **12**, and **13**) is stronger. Accordingly, we cannot anticipate how the selectivity for systems B and C alters with temperature.

Hydrophobicity (the strength of hydrophobic interaction)[5)] is reported to increase progressively with increasing temperature. Considering the above discussions, however, it is uncertain whether or not the temperature dependence of the selectivity in water — at least for systems B and C (Fig. 5) — can be explained by hydrophobic interaction.

There are many examples where hydrophobic interaction is not the factor controlling the selectivity (r).[6a,6c,7,16)].

A Possible Explanation of Changes in Pattern of Temperature Dependence of Recognition. Intermolecular association would be responsible for the decrement in the selectivity in alcohols with increasing temperature. Hydrophobic interaction might be one factor affecting the increment in the selectivity in water with increasing temperature.

Reactivity difference between thiols **1** and **2** cannot explain the observed selectivity. The physicochemical properties of the solvents would affect the selectivity, but any one of them cannot explain it by itself.

Though not discussed above, shape-specific weak interactions[27)] between R^1 and R^2 would influence the selectivity because of their ability to control molecular recognition.[7)]

Results and discussions described above suggest that specific solvent structures[28,29)] would be formed, through hydrogen bond network among solvent molecules, around the tetramers, which would affect weak interactions between the recognition sites, thus leading to changes in the selectivity. Detailed solvent structures remain unknown.

Conclusion

The present work has demonstrated that (i) in *alcohols*, the selectivity *decreases* (or increases in one case) markedly with increasing temperature and (ii) in *water*, the selectivity *increases* with increasing temperature. This indicates that amplification effect in molecular recognition is not observed in alcohols or water. It is striking that addition of a certain amount of an alcohol to water leads to specific molecular recognition.

It is hoped that detailed solvent structures (i.e., those of aqueous alcohols as well as alcohols and water) around the tetramers will be made clear in the near future using novel techniques such as those[28b,29a)] recently developed.

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 Model 204 system including a UV detector attached to a Waters 741 Data Module (integrator).

Materials: The alcohols were of the practically best grade available [MeOH and *i*-PrOH (Wako, JIS Special Grade) and EtOH and *n*-PrOH (Wako, Special Grade)] and were used as such. Water was purified through Millipore Milli-Q water purification system followed by distillation.

Preparation of Thiols: 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.[30] The reaction mixtures were concentrated in vacuo, washed with water in order to remove the co-existing acetylated cysteamine [$MeC(=O)$-$NHCH_2CH_2SH$] because of high solubilities of the resulting thiols in MeCN used as a solvent, dried in vacuo, and recrystallized from argon-saturated diethyl ether–hexane. Thiols **1** had the following properties [^{1}H NMR (270 MHz) spectra of **1** were measured in $CDCl_3$ at 0.02 mol dm^{-3}].[1,31,32]

1c: Mp 88.0—88.5 °C (diethyl ether–hexane); ^{1}H NMR (270 MHz, $CDCl_3$) δ=0.90 (3H, t, J=7 Hz), 1.19—1.39 (16H, m), 2.09 (1H, t, J=9 Hz, SH), 3.27—3.37 (4H, m, SCH_2 and NCH_2), 8.30 (1H, s) and 9.55 (1H, s); MS *m/z* 274 (M^+). Found: C, 56.6; H, 9.3; N, 10.2; S, 12.2%. Calcd for $C_{13}H_{26}N_2O_2S$: C, 56.91; H, 9.55; N, 10.21; S, 11.67%.

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[16] and had the following properties [^{1}H NMR (270 MHz) spectra of **2** were measured in $CDCl_3$ at 0.02 mol dm^{-3}].[1,31]

2c: Mp 85.0—85.5 °C (diethyl ether–hexane); ^{1}H NMR (270 MHz, $CDCl_3$) δ=0.88 (3H, t, J=7 Hz), 1.23—1.36 (16H, m), 1.42 (1H, t, J=9 Hz, SH), 2.32 (2H, t, J=7 Hz, $COCH_2$), 2.64—2.77 (2H, m, SCH_2), 3.45—3.55 (2H, m, NCH_2), 8.88 (2H, s). Found: *m/z* 289.1950. Calcd for $C_{14}H_{29}N_2O_2S$: M+1, 289.1851.

Preparation of Disulfides: 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 either by repeated recrystallization of the corresponding oxidation mixtures in cases where the *r* for a given system was larger than ca. 1 or by reaction of the corresponding disulfides [2,4-$(NO_2)_2C_6H_3S$–$SCH_2CH_2NHC(=O)NHC(=O)$–R^2] with thiols **1** in the presence of silver acetate in DMF under argon as described previously[33] followed by recrystallization of the corresponding reaction mixtures in cases where the *r* for a given system was smaller than ca. 1. Disulfides **3** and **4** had the following properties [^{1}H NMR (270 MHz) spectra were measured in $(CD_3)_2SO$ at 0.01 mol dm^{-3}].[1,31,34]

3c **(R^1 = *n*-$C_{10}H_{21}$):** Mp 154.0—155.5 °C (THF–dichloromethane); ^{1}H NMR (270 MHz, $CDCl_3$) δ=0.88 (6H, t, J=7 Hz), 1.29—1.39 (32H, m), 3.12—3.20 (4H, m, NCH_2), 3.65(4H, s, SCH_2CO), 8.30 (2H, s), 10.41 (2H, s); MS *m/z* 546 (M^+). Found: C, 57.0; H, 9.0; N, 10.5; S, 11.7%. Calcd for $C_{26}H_{50}N_4O_4S_2$: C, 57.12; H, 9.22; N, 10.25; S, 11.60%.

4bb **(R^1=R^2= *n*-C_5H_{11}):** Mp 178.5—180.0 °C (THF–dichloromethane); ^{1}H NMR (270 MHz, $CDCl_3$) δ=0.83—0.91 (6H, m), 1.17—1.57 (12H, m), 2.26 (2H, t, J=7 Hz), 2.87 (2H, t, J=6 Hz), 3.12—3.20 (2H, m, $SC\underline{H}_2CH_2N$), 3.30—3.68 (4H, m, $SCH_2C\underline{H}_2N$ and $SC\underline{H}_2CO$), 8.56 (1H, s), 10.30 (2H, s), 10.44 (1H, s). Found: *m/z* 421.1931. Calcd for $C_{17}H_{33}N_4O_4S_2$: M+1, 421.1954.

4cc **(R^1=R^2= *n*-$C_{10}H_{21}$):** Mp 168.0—169.0 °C (THF–dichloromethane); ^{1}H NMR (270 MHz, $CDCl_3$) δ=0.83—0.91 (6H, m), 1.17—1.57 (32H, m), 2.26 (2H, t, J=7 Hz), 2.87 (2H, t, J=6 Hz), 3.12—3.20 (2H, m, $SC\underline{H}_2CH_2N$), 3.30—3.68 (4H, m, $SCH_2C\underline{H}_2N$ and $SC\underline{H}_2CO$), 8.56 (1H, s), 10.30 (2H, s), 10.44 (1H, s). Found: *m/z* 561.3501. Calcd for $C_{27}H_{53}N_4O_4S_2$: M+1, 561.3412.

4da **(R^1= Ph and R^2= *n*-C_4H_9):** Mp 170.0—172.0 °C (THF–dichloromethane); ^{1}H NMR (270 MHz, $CDCl_3$) δ=0.85 (3H, t, J=7 Hz), 1.21—1.30 (2H, m), 1.43—1.54 (2H, m), 2.27 (2H, t, J=7 Hz, $COC\underline{H}_2CH_2$), 2.92 (2H, t, J=6 Hz, $SC\underline{H}_2CH_2N$), 3.46—3.53 (2H, m, $SCH_2C\underline{H}_2N$), 3.71 (2H, s, $SC\underline{H}_2CO$), 8.58 (1H, s), 10.30 (2H, s), 10.83 (1H, s); MS *m/z* 412 (M^+). Found: C, 49.25; H, 5.80; N, 13.66; S, 15.63%. Calcd for $C_{17}H_{24}N_4O_4S_2$: C, 49.51; H, 5.86; N, 13.59; S, 15.52%.

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 the use of their absorption (i) at 225 nm for systems A, B, and C and (ii) at 251 nm for system D, after separation of the three disulfides in the mixture by HPLC using LiChrosorb CN with hexane–*i*-PrOH (96 : 4) as an eluent. The *r* values given in Figs. 1, 2, 3, 4, 5, and 6 represent the mean values of two or more experiments, and were reproducible within the errors shown therein.

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