Methylation of a Benzene Ring as a Chemical Signal. Marked Changes in the Pattern of Temperature Dependence of the Selectivity in Oxidation of a Pair of Associating Thiols

Tadashi Endo,* Akihiro Okubo, Yuji Kaneko, Masatoshi Uehara,
Hidetoshi Tasai, Akiyoshi Sato, Kunio Nikki,†
Naoya Nakagawa,† and Shinichi Kamei††
Department of Chemistry, College of Science and Engineering, Aoyama Gakuin University, Chitosedai, Setagaya-ku, Tokyo 157
† *The University of Electro-communications, Chofugaoka, Chofu, Tokyo 182*
†† *Kyowa Kagaku Co., Ltd., 3-4-5, Hongo, Bunkyo-ku, Tokyo 113*

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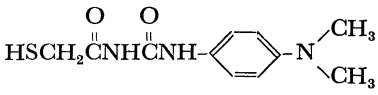
Temperature dependence of the selectivity [the ratio of an unsymmetrical disulfide to symmetrical one] has been studied in oxidation of a pair of associating thiols, each with the recognition site [$C_6H_4N(CH_3)_2$ and R^1], with O_2 in 80% (v/v) acetonitrile–20% water in the range of −10 to 70 °C. The selectivity for $R^1=C_6H_4CH_3$ shows a minimum at 35 °C, whereas for $R^1=C_6H_5$ the selectivity exhibits a maximum at 35 °C; for $R^1=C_6H_4C_2H_5$ plots of the selectivity against temperature shows a sigmoid-like curve. The selectivity in oxidation of a pair of non-associating thiols, 2-(dimethylamino)ethanethiol and a para-substituted benzenethiol, remains practically unaltered with temperature in the same solvent. Though thiol-disulfide exchange reaction proceeds slowly below 35 °C, the selectivity in the exchange differs entirely from that in the oxidation. It has been found that changes in hydrophobicity with R^1 and temperature cannot explain dramatic alterations in the selectivity with R^1 and temperature.

Highly specific molecular recognition underlies living systems. Proteins such as restriction endonucleases[1)] and modification methylases[1)] can recognize the methyl group. The enzyme-catalyzed methylation of DNA,[2)] proteins,[3)] and phospholipids[4)] is among the biological control mechanisms,[5)] and has been investigated extensively: (1) methylation of nucleotide bases (5-methylcytosine and *N*⁶-methyladenine) lying within the recognition sequence serves to protect the cell's own DNA from being degraded by its restriction endonucleases,[6)] (2) methylation, for example, of cytosine is involved in gene regulation and differentiation,[7)] (3) most eukaryotic, but not bacterial, messenger RNAs contain a methylated guanosine (7-methylguanosine) —— the "cap" —— at their 5′-ends,[8)] and (4) methylation of protein-carboxyl groups (*e.g.*, γ-carboxyl group(s) of glutamic acid residue(s)) is associated with the control of chemotactic behavior and with signal transduction in both prokaryotes and eukaryotes.[9)]

It is striking that biological systems make use of quite a "simple" chemical modification, *methylation*, in their control mechanisms as described above. In spite of biological significance, the problem of methylation has not so far been examined from a chemical point of view of, for example, what influence the methylation exercises on chemical events as well as of how noncovalent weak interactions are altered upon methylation. We report here that the methylation of a benzene ring remote from the reaction site radically alters the pattern of temperature dependence of the selectivity in oxidation of a pair of associating thiols.

In this study we used two reaction systems: the associating system (system A) and non-associating system (system N). These two systems are structurally related to each other except for the ability to associate. System A consists of a pair of thiols (**1** and **2**)[10)] which can associate mainly through intermolecular hydrogen bonding.[11)] Thiols **1** and **2** each have the three sites —— thereaction site (SH group) where model reaction takes place, the binding site ($-\overset{\text{O}}{\overset{\|}{\text{C}}}\text{NH}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{NH}-$, acylurea bond) that can participate in intermolecular hydrogen bonding,[11)] and the recognition site [$C_6H_4N(CH_3)_2$ or R^1] that can participate in the selective recognition. System A is further divided into system A_p (**1** and **2p**), system A_t (**1** and **2t**), system A_e (**1** and **2e**), and system A_a (**1** and **2a**). System N is made up of a pair of thiols, 2-(dimethylamino)ethanethiol (**3**) and a para-substituted benzenethiol (**4**); this system is also divided into system N_p (**3** and **4p**) and system N_t (**3** and **4t**).



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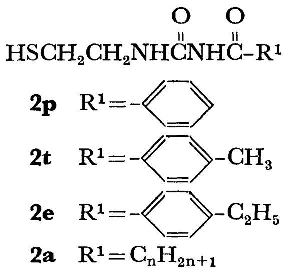
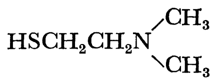


$$\text{HSCH}_2\text{CH}_2\text{NH}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{NH}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{-R}^1$$

2a $R^1=C_nH_{2n+1}$



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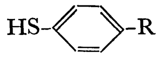
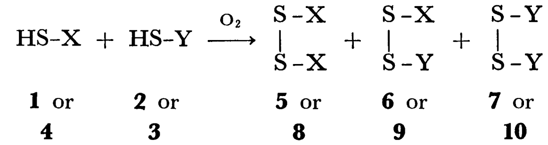


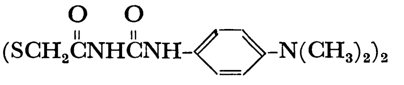
4p $R^2=H$
4t $R^2=CH_3$

As a model reaction, oxidation of system A or N with oxygen was chosen. Oxidation of system A gives three disulfides, two symmetrical disulfides (**5** and **7**) and an unsymmetrical disulfide (**6**) as shown in Eq.

1; similarly, oxidation of system N gives the corresponding three disulfides (**8**—**10**) (Eq. 1). The determination of the products in the case of oxidation of system A was made according to our method[12)] which took advantage of separation by thin-layer chromatography (TLC) and UV absorption due to the moiety $\text{-HNC}_6\text{H}_4\text{N(CH}_3)_2$.[13)] The selectivity ($R$) —— a measure of *molecular recognition* —— in this oxidation is represented by the ratio of the yield of **6** (or **9**) to that of **5** (or **8**) [R=**6**/**5** for system A or **9**/**8** for system N].[13,14)]



$$\underset{\mathbf{1}\text{ or }\mathbf{4}}{\text{HS-X}} + \underset{\mathbf{2}\text{ or }\mathbf{3}}{\text{HS-Y}} \xrightarrow{O_2} \underset{\mathbf{5}\text{ or }\mathbf{8}}{\begin{array}{c}\text{S-X}\\|\\\text{S-X}\end{array}} + \underset{\mathbf{6}\text{ or }\mathbf{9}}{\begin{array}{c}\text{S-X}\\|\\\text{S-Y}\end{array}} + \underset{\mathbf{7}\text{ or }\mathbf{10}}{\begin{array}{c}\text{S-Y}\\|\\\text{S-Y}\end{array}} \tag{1}$$



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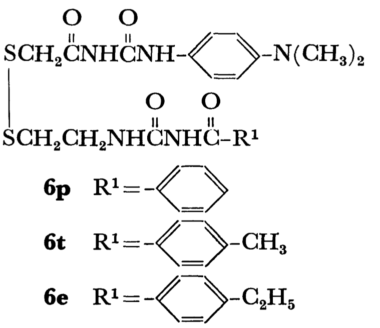
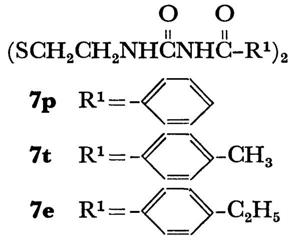




Results

Selectivity in Oxidation of a Pair of Thiols. The temperature dependence of the selectivity (R) in oxidation of system A was examined in 80% (v/v) acetonitrile–20% water within the temperature range from −10 to 70 °C. The results are shown in Table 1 and Fig. 1: (1) each of the three curves for systems A_p, A_t, and A_e shows considerable symmetry around 35 °C; (2) the selectivity for all the three systems increases in going from 0 to −10 °C; (3) even at 70 °C the R values do not approach 2 (the statistically expected value[15)] for this type of reaction) except in the case of system A_p, this result contrasting sharply with the fact[16)] that for system A_a where R^1=i-C_5H_{11} and n-C_5H_{11} the R values at 70 °C were 2.9 and 2.3, respectively.

Control experiments were performed at 20, 35, and 70 °C using oxidation of system N. The R values were found to vary only slightly (*i.e.*, from 1.6 to 1.8) in both systems N_p and N_t within this temperature

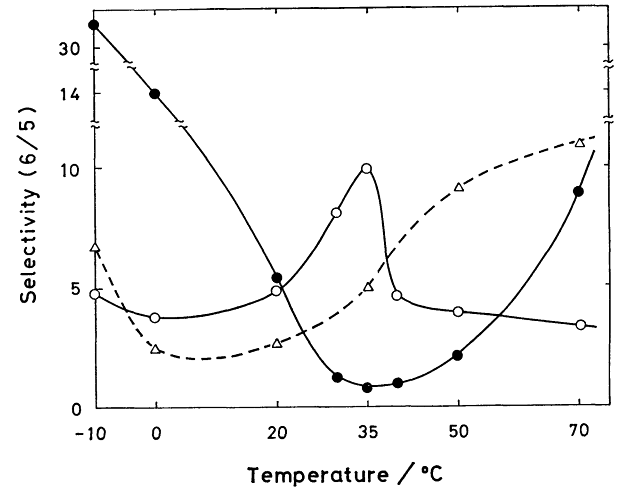


Fig. 1. Temperature dependence of the selectivity (**6**/**5**) in oxidation of system A with O_2 in 80% (v/v) CH_3CN–20% H_2O.
○: System A_p (**1**+**2p**), ●: system A_t (**1**+**2t**), △: system A_e (**1**+**2e**).

TABLE 1. YIELDS OF DISULFIDES (**5** AND **6**) AND THE SELECTIVITY IN OXIDATION[a)] OF SYSTEM A WITH O_2

Temp/°C	System A_p (**1**+**2p**) Yield[b)]/% **5**	**6**	Selectivity (**6**/**5**)	System A_t (**1**+**2t**) Yield[b)]/% **5**	**6**	Selectivity (**6**/**5**)	System A_e (**1**+**2e**) Yield[b)]/% **5**	**6**	Selectivity (**6**/**5**)
−10	14	67	4.8	2.9	89	31	11	75	6.8
0	16	61	3.8	5.9	84	14	22	54	2.5
20	14	68	4.9	13	70	5.4	21	56	2.7
30	9.5	77	8.1	30	38	1.3			
35	8.1	81	10	34	29	0.85	14	70	5.0
40	14	66	4.7	32	33	1.0			
50	16	64	4.0	22	46	2.1	8.6	79	9.2
70	18	61	3.4	9.0	80	8.9	7.5	81	11

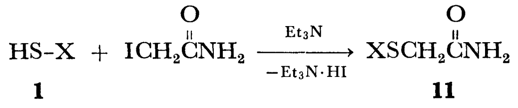
a) The time required for the oxidation was 1.5 h for 70 °C, 3 h for 35 °C, and 50 to 70 h for 0 °C. b) Yields are calculated in such a way that (1) when **1** and **2** are converted exclusively to **5** and **7**, respectively, the yields of **5** and **7** are both 50% and (2) when **1** and **2** are converted exclusively to **6**, the yield of **6** is 100%.

range.

Selectivity in Thiol-Disulfide Exchange Reaction. We studied thiol-disulfide exchange reaction, known to take place generally as shown in Eqs. 2 and 3,[17] in order to elucidate whether or not the exchange affects the experimental results illustrated in Fig. 1. The exchange was carried out mainly between thiol **2** and disulfide **5** at 0, 35, and 70 °C for the same time as with the corresponding oxidation. Since resulting thiol **1** (Eqs. 2 and 3) could not be separated from disulfide **5** by TLC in the solvent required to separate the three disulfides **5**—**7** from one another, **1** was converted to the corresponding sulfide having the carbamoyl group (**11**), by treatment with iodoacetamide and Et_3N (Eq. 4),[18] so as to be determined by the method[12] described above.

$$\underset{\mathbf{2}}{\text{HS-Y}} + \underset{\mathbf{5}}{\text{XS-SX}} \rightleftharpoons \underset{\mathbf{6}}{\text{XS-SY}} + \underset{\mathbf{1}}{\text{HS-X}} \qquad (2)$$

$$\underset{\mathbf{2}}{\text{HS-Y}} + \underset{\mathbf{6}}{\text{XS-SY}} \rightleftharpoons \underset{\mathbf{7}}{\text{YS-SY}} + \underset{\mathbf{1}}{\text{HS-X}} \qquad (3)$$



$$\underset{\mathbf{1}}{\text{HS-X}} + ICH_2\overset{O}{\overset{\|}{C}}NH_2 \xrightarrow[-Et_3N\cdot HI]{Et_3N} \underset{\mathbf{11}}{XSCH_2\overset{O}{\overset{\|}{C}}NH_2} \qquad (4)$$

In Table 2 are listed the experimental data: (1) at 0 °C the exchange reaction is slow in all cases; (2) at 35 °C the exchange proceeds to some extent except in the case of **2t** and **5**; (3) at 70 °C the reaction is fast; (4) the rate of the reaction increases progressively with increasing temperature as exemplified by the yield of **5**.

The exchange was also performed between thiol **1** and disulfide **7** for the same time as with the corresponding oxidation (Table 2). In view of the results with the exchange between **2** and **5**, the reaction temperature to be examined was limited to 35 and 70 °C, and disulfide **7** to **7p** and **7t**. As a result, 75 to 94% of **1** was found to remain unreacted in all cases. From the data shown in Tables 1 and 2, it has been demonstrated that the selectivity in the exchange differs entirely from the selectivity in the oxidation.

Table 2. Yields of **5**, **6**, and **11** and the selectivity in thiol-disulfide exchange reaction[a)]

Temp/°C	Reactant	Yield[b)]/% 5	6	11	Selectivity (6/5)
0	**2p**+**5**	42	≃0	9.5	≃0
0	**2t**+**5**	33	24	4.9	0.73
0	**2e**+**5**	33	25	5.7	0.76
35	**2p**+**5**	20	22	31	1.1
35	**2t**+**5**	9.0	43	33	4.8
35	**2e**+**5**	27	22	22	0.81
70	**2p**+**5**	≃0	10	87	≃∞
70	**2t**+**5**	3.7	21	65	5.7
70	**2e**+**5**	2.5	32	59	13
35	**1**+**7p**	4.5	13	75	2.9
35	**1**+**7t**	2.5	3.8	88	1.5
70	**1**+**7p**	≃0	11	87	≃∞
70	**1**+**7t**	0.6	1.0	94	1.7

a) The exchange reaction time was the same as with the oxidation; see footnote a in Table 1. b) The yields of **5** and **6** are calculated as in Table 1. The yield of **11** is calculated in such a way that when **5** is converted exclusively to **1**, the yield of **11** is 100%.

Further, we examined disulfide-disulfide exchange reaction between **5** and **7t** at 35.0 °C in the presence of Et_3N, but in the absence of a thiol, for 3 h (Eq. 5). The exchange proved not to occur practically, as indicated by the observation that recovery of **5** was 92% with the yield of **6t** being <1%; this agrees well with the generally accepted view.[17a)]

$$\underset{\mathbf{5}}{\text{XS-SX}} + \underset{\mathbf{7t}}{\text{YS-SY}} \rightleftharpoons \underset{\mathbf{6t}}{2\,\text{XS-SY}} \qquad (5)$$

Physicochemical Properties of the Associating Thiols. The degree to which molecules self-associate in solution can be represented, for example, by the "degree of association" (f): the f value for the solute is obtained by dividing the stoichiometric mole fraction of the solute by the effective mole fraction of the solute.[19] The data (in benzene at 36.0 °C) in Table 3 exhibit that thiols **1** and **2** are similar in f values to each other and that f values for **2** increase with increasing number of the carbon atoms introduced at the para position of its benzene ring.

The solubilities of **1** and **2** in aqueous acetonitrile, the same solvent as in the oxidation, in the range of 20 to 50 °C are illustrated in Fig. 2 and partly in Table 3 for comparison with the melting points of **1** and **2**. Figure 2 shows that (1) the solubility increases with increasing temperature for each thiol, (2) the solubility of **2** increases in the order **2t**<**2e**<**2p** at all temperatures studied, and (3) **1** is approximately equal in solubility to **2t** at all temperatures. There exists an empirical rule that solubilities decrease with increasing melting-points for a series of homologs.[20]

Table 3. Physicochemical properties of thiols **1** and **2**

Thiol	f[a)]	Solubility[b)] (35 °C)	Mp[c)] θ_m/°C	Chemical shift[d)] H^α (or H^γ)	H^β (or H^δ)
1	1.31	3.06	173	9.50	10.17
2p	1.30	13.4	148	8.73	8.85
2t	1.33	3.15	187	8.58	9.02
2e	1.41	5.36	165	8.56	8.92

a) The values at 36.0 °C and 0.010 M (1 M = 1 mol dm^{-3}) in benzene. For the definition, see the text. b) Expressed in mmol per 100 g of 80% (v/v) CH_3CN-20% H_2O. c) Obtained from the DTA curves. d) The chemical shifts for the individual thiols in $CDCl_3$ at 34 °C and 0.020 M. The values are in ppm downfield from TMS. Greek letters (α, β, γ, and δ) indicate assignments as given in Fig. 4. When **1** and **2** were mixed together in equimolar amounts (0.010 M each) in $CDCl_3$ at 34 °C, the chemical shifts of the NH^α proton were 9.39, 9.36, and 9.38 ppm for systems A_p, A_t, and A_e, respectively, those of the NH^γ proton being 8.67, 8.58, and 8.58 ppm for systems A_p, A_t, and A_e, respectively.

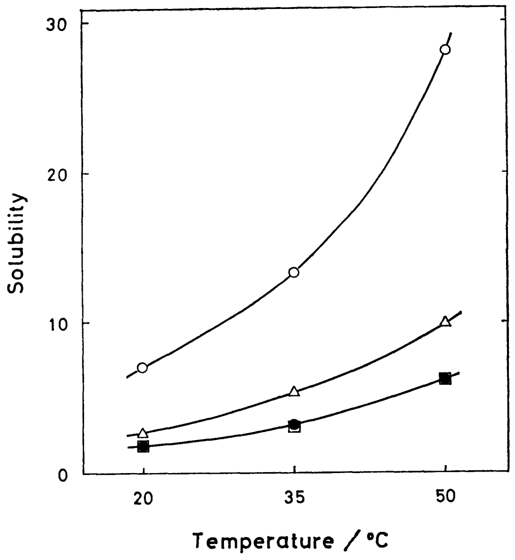


Fig. 2. Temperature dependence of the solubility for **1** and **2**. The solubilities are expressed in mmol/100 g of 80% (v/v) CH_3CN-20% H_2O.
□: **1**, ○: **2p**, ●: **2t**, △: **2e**.

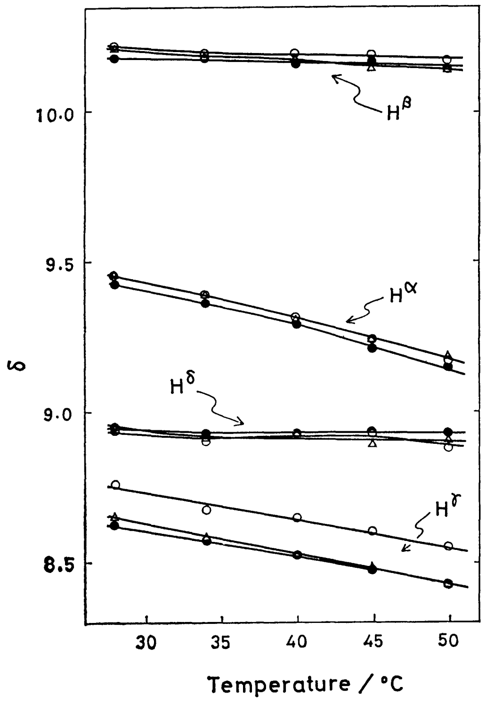


Fig. 3. Temperature dependence of the NH proton chemical shifts for a 1:1 mixture of **1** and **2** (0.010 M each) in $CDCl_3$ at 34 °C.
○: System A_p (**1**+**2p**), ●: system A_t (**1**+**2t**), △: system A_e (**1**+**2e**). For Greek letters, see Fig. 4.

The data in Table 3 show that this rule holds true for thiol **2**.

Using surface tension measurement, we have examined whether or not system A forms micelles in aqueous acetonitrile. As a result, it has been found that in 80% (v/v) acetonitrile–20% water surface tension for systems A_p, A_t, and A_e all remains constant (32.0±0.2 dyn/cm) over the concentration range of 1.3×10^{-6} to 1.0×10^{-2} M.[21]

Figure 3 provides the temperature dependence of the NH proton chemical shifts for system A in $CDCl_3$. The results shown in Fig. 3 reveal that the chemical shifts are more sensitive to temperature for the NH^α and NH^γ protons (Fig. 4), each participating in intermolecular hydrogen bond, than for the NH^β and NH^δ protons (Fig. 4), each participating in intramolecular hydrogen bond. Furthermore, chemical shift changes with the structure of R^1 proved to be larger for the NH^γ proton than for the NH^α proton.

Discussion

The most striking feature of the results reported above is the radical changes in temperature-selectivity profiles from one pattern to another around 35 °C —— from a maximum-having curve to minimum-having one (or to sigmoid-like one) —— on methylation (or ethylation) of the benzene ring in **2** at the para position.[22] This observation would imply that methylation of a benzene ring can function as a *chemical signal* (*or information*) in an associating system. In what follows, we would like to discuss some factors which have the possibility of affecting the temperature dependence of the selectivity on methylation and ethylation.

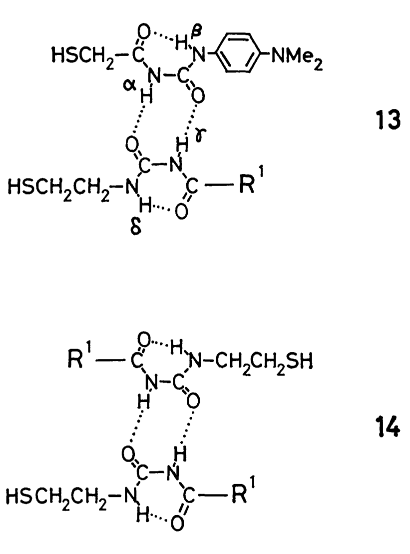


Fig. 4. Association patterns of three dimers **12**—**14**.

Intermolecular Association. Table 3 clarifies that **1** and **2** associate strongly with themselves in benzene. In Fig. 4 are given the association patterns[23] of three dimers, two homodimers (**12** and **14**) and a heterodimer (**13**), the association patterns being assigned mainly on the basis of the NMR results (Fig. 3) as in our previous work.[11] Moreover, our study

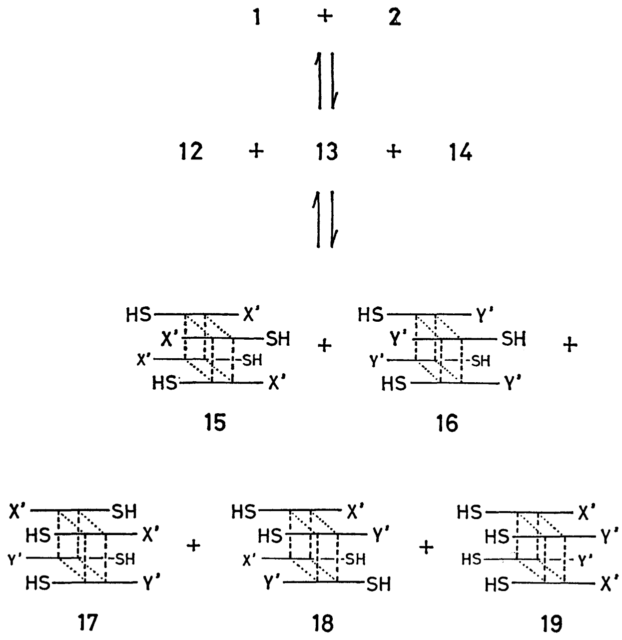
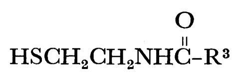


Fig. 5. Association schemes of tetramers **15**—**19** formed by dimerization of dimers **12**—**14**.
……: Hydrogen bond responsible for stabilization of dimers, ----: noncovalent weak interactions responsible for stabilization of tetramers. The symbols X′ and Y′ represent $C_6H_4N(CH_3)_2$ and R^1, respectively.

has indicated that dimers **12**—**14** would dimerize to form tetramers (Fig. 5) —— two homotetramers (**15** and **16**) and three heterotetramers (**17**—**19**)[23,24] —— in equilibrium with dimers **12**—**14** and monomers (**1** and **2**) and that oxidation of system A_a proceeds through these tetramers **15**—**19**. Since the f value of 1.41 for **2a** (R^1=i-C_5H_{11}) is similar to those for **2p**, **2t**, and **2e** under the same conditions as in Table 3, it seems reasonable to assume that oxidation of systems A_p, A_t, and A_e proceeds also through tetramers **15**—**19**. There is substantial evidence for the presence of tetramers[25] and for their participation in biological reactions.[11]

Simple thiols are reported to be capable of forming very weak hydrogen bonds with themselves.[26] However, considering that f values even for the amide derivatives of **2** (**20**) which can form a single hydro-



$HSCH_2CH_2NH\overset{O}{\overset{\|}{C}}\text{-}R^3$

20

gen bond are less than 1.05 in benzene at 36.0 °C and 0.010 M,[27] it is probably fair to say that thiols **3** and **4** are present mainly as monomers in dilute solutions.[26]

The selectivity remained approximately constant, when oxidation of system N was performed at 20, 35, and 70 °C. This finding together with the data in Fig. 1 demonstrates the importance of intermolecular association in molecular recognition, in agreement with our previous conclusion.[16]

Seeman *et al.* studied how the base pairs in double helical nucleic acids could be recognized by proteins, and concluded that using two hydrogen bonds, fidelity of base pair recognition might be achieved, whereas a single hydrogen bond was inadequate for uniquely identifying any particular base pair.[28]

Possibility of Micelle Formation in Aqueous Acetonitrile. It is recognized that when the concentration of a surfactant in an aqueous solution is increased, surface tension is initially reduced with its concentration, for example, to *ca.* 25 dyn/cm,[29] and then kept approximately constant beyond the critical micelle concentration (cmc).[30] In contrast, surface tension of system A in aqueous acetonitrile did not alter over the concentration range of 1.3×10^{-6} to 1.0×10^{-2} M, the latter corresponding to the concentration of a nearly saturated solution. This result clearly demonstrates that no micelle formation occurs for system A in the same solvent as in the oxidation.

Solubility Differences and Heterogeneity of Reactions. System A was homogeneous at higher temperatures in 80% (v/v) CH_3CN-20% H_2O under argon in the presence of a catalytic amount of Et_3N: system A_p was completely soluble above 40 °C, and systems A_t and A_e above 50 °C. Accordingly, differential solubilities of **1** and **2** at lower temperatures may influence the selectivity in oxidation of system A in such a way that the larger the solubility differences between **1** and **2** are, the smaller the R values become. Since solubility differences between **1** and **2** vary monotonously with temperature, the temperature dependence of solubility differences between **1** and **2** has no appreciable correlation with that of the selectivity for system A (Figs. 1 and 2).

Rys reported the mixing effects on the product distribution in competitive consecutive or parallel reactions.[31] This problem was not examined directly in the present case. However, if diffusion effects influence the selectivity, the temperature-selectivity profiles for systems A_p, A_t, and A_e all should become similar in pattern to one another as temperature increases. As is clear from Fig. 1, this does not hold true, suggesting that the selectivity would not be disguised by diffusion effects.

Exchange Reactions. It is generally accepted that thiol-disulfide exchange reaction occurs easily if a base is present and that the rate of the exchange increases with temperature.[17c] In contrast, in this study thiol-disulfide exchange has been found to take place to a small extent below 35 °C (Table 2), and no appreciable disulfide-disulfide exchange has occurred. It seems probable, therefore, that the exchange reactions have not so great influence on the selectivity in the oxidation of system A at least below 35 °C, judging from the fact that (1) there is a remarkable difference in the selectivity between the exchange and the oxidation (Tables 1 and 2) and (2) the amount of the thiols which can participate in thiol-disulfide exchange is reduced as the oxidation proceeds.

Hydrophobic Interaction.[32] The strength of hydrophobic interaction (hydrophobicity)[33] appears to increase with increasing surface area[34] of the nonpolar group removed from contact with water:[35] The $\Delta G°$ value for the transfer of solute from the pure liquid to aqueous solution increases in the order benzene (4.61 kcal/mol) < toluene (5.33 kcal/mol) < ethylbenzene (6.07 kcal/mol).[36] Furthermore, hydrophobicity represented by ΔG° for the transfer is known to increase progressively with temperature.[36]

The temperature dependence of R described above was investigated in acetonitrile containing a given amount (20% (v/v)) of water. In addition, using acetonitrile with varying amounts (10 to 50% (v/v)) of water as solvents, oxidation of system A_p was performed similarly at 35.0 °C; R values were plotted against the volume percent of water. As a result, R values were found to show a maximum at 20% of water.[37] If hydrophobic interaction is responsible for the selectivity changes with temperature on methylation and ethylation, the R values for all of systems A_p, A_t, and A_e should vary progressively with volume percent of water and with temperature because of progressive increment of hydrophobicity with these two factors. This does not coincide with the experimental results. These arguments indicate that marked changes in the selectivity with temperature on methylation and ethylation (Fig. 1) cannot be interpreted at all in terms of hydrophobic interaction.

Evidence is now presented to force reexamination of the concept of hydrophobic interaction.[38–40] Lawaczeck and Wagner[38] pointed out that this interaction, though consistent with the behavior of proteins or nonpolar amino acids, failed to account for the thermodynamic data in the nucleic acid field —— the large positive entropy values obtained for nucleic acid melting and for transfer of nucleobases from organic solvents to water. Cramer III[39] showed that hydrophobic interaction was incompatible with relationships between the structures of various nonpolar solutes and their solvation energies by water and 1-octanol. Moreover, based on adhesion and surface free energy data to indicate that the forces between hydrocarbons and water in contact with each other are attractive and not repulsive, Hildebrand[40] has argued against the use of the word "hydrophobic" to describe the relative lack of attraction between water and nonpolar substances.

Three-dimensional Shape-specific Weak Interactions. We have recently suggested that weak interactions specific for three-dimensional shape of nonpolar groups are responsible for selective molecular recognition in oxidation of system A_a.[41] This type of interactions are assumed to possess the following features: (1) they have energies smaller than that for hydrogen bond, (2) they are made up of individual interactions between groups, between a group and an atom, or between atoms, and (3) they can reflect three-dimensional shape of nonpolar groups such as alkyl groups.[42]

The data in Fig. 3 indicate that there is inappreciable difference in the pattern of temperature dependence of the NH^α proton chemical shifts on methylation and ethylation. Hence it seems difficult to consider that specific weak interactions[43] are reflected in the NH^α proton resonance.

Abraham reported that the major contribution to the hydrophobic CH_2 free-energy increment ($\Delta G^\circ=$ 0.54 kcal/mol at 298 K) was an enthalpic effect ($\Delta H^\circ=$ 0.46 kcal/mol) and not an entropic effect ($-T\Delta S^\circ=$ 0.08 kcal/mol), by dissecting the gas→water CH_2 contribution into a favorable normal solvent effect and a true (unfavorable) hydrophobic effect.[44] Abraham's conclusion stimulates our view that the selectivity changes on methylation are better to understand by three-dimensional shape-specific weak interactions rather than by hydrophobic interaction.

Conclusion

The pattern of temperature dependence of the selectivity in oxidation of a pair of associating thiols varies radically from a maximum-having curve to a minimum-having one (or to a sigmoid-like one) upon methylation (or ethylation) of a benzene ring remote from the reaction site. This is not the case with the oxidation of a pair of non-associating thiols. These experimental results would mean that methylation of a benzene ring can function as a *chemical signal* in an associating system.

Further, any of the factors discussed in this work —— solubility differences, exchange reactions, hydrophobic interaction, and three-dimensional shape-specific weak interactions —— alone cannot, at present, satisfactorily explain the dramatic alterations in the selectivity with temperature on methylation and ethylation.

Experimental

Determination of Physicochemical Properties of Thiols and Disulfides. Melting points were obtained from the DTA (Differential Thermal Analysis) curves recorded with a Shimadzu DT-20B thermal analysis apparatus. The samples were finely powdered, diluted with fine-grained quarz wool in the specimen holder, and heated in a stream of nitrogen with a heating rate of 10 °C/min.

The effective mole fraction of the solute required to calculate the degree of association (f) was determined on a Hitachi Perkin-Elmer 115 molecular weight apparatus using a differential vapor pressure method at 36.0 °C and 0.010 M in benzene.

The solubility was measured gravimetrically by evaporating a saturated solution of **1** or **2** to dryness under reduced pressure. The saturated solution of **1** or **2** was prepared by vigorous stirring of the two-phase mixture for 8 h in a well-stirred water bath held at constant temperatures controlled to ±0.1 °C. Further stirring caused no increase in solubility. Separate experiments containing different ratios of residual solid solute to solvent yielded the same equilibrium solubility. The solubility recorded for a given temperature is the mean value of at least four measurements which did not deviated more than 4%.

Surface tension for three systems A_p, A_t, and A_e was measured on a Kyowa surface tensiometer CBVP-A3 using a counter balance and vertical plate method in 80% (v/v) CH_3CN–20% H_2O at 25 °C. The concentrations of each thiol employed for this measurement were 1.3×10^{-6}, 2.6×10^{-5}, 5.2×10^{-4}, and 1.0×10^{-2} M; the concentration of 0.010 M corresponds to that of a nearly saturated solution of system A. The uncertainty in surface tension is ±0.2 dyn/cm.

^{1}H NMR spectra were measured on a Hitachi R-20B spectrometer at 60 MHz. The chemical shifts for the NH protons shown in Fig. 3 were measured precisely by the sideband method with the aid of a frequency counter. The uncertainty in chemical shifts is ±0.01 ppm.

Materials. Acetonitrile was dried and purified by distillation from calcium hydride and phosphorus pentaoxide, and stored over molecular sieves. Water was distilled after deionization. Precoated TLC plates (silica gel 60 F_{254}) were purchased from Merck Darmstadt.

Thiol **1** was prepared as described before[45] and had the following properties (the melting point and NH proton chemical shifts are listed in Table 3): R_f 0.69 [silica, Et_2O–$EtCO_2H$–MeOH (30:3:1) and Et_2O–$EtCO_2H$–MeOH–AcOEt (60:6:1:1)]; NMR ($CDCl_3$) δ=2.10 (1H, t, J=9.0 Hz, SH), 2.94 (6H, s, $N(CH_3)_2$), 3.38 (2H, d, J=9.0 Hz, $HSCH_2$), 6.6—7.6 (4H, m, ArH). Found: C, 52.45; H, 6.10; N, 16.64; S, 12.69%; M^+, 253.

Thiol **3** was prepared by reaction of its hydrochloride (Tokyo Kasei Kogyo Co., Ltd.) with an excess of tetraethylenepentamine and purified by distillation (bp 57—58 °C (80 mmHg**)); satisfactory spectral data were used to support the assignment of **3**. Thiol **4** was obtained from Tokyo Kasei Kogyo Co., Ltd. and purified by distillation before use. Thiol **20** was synthesized as reported before.[27]

*Preparation of Thiol **2**.* Freshly sublimated cysteamine (10 mmol) was dissolved in 35 ml of tetrahydrofuran (THF) under argon by heating. This solution was cooled to 0 °C. To the cold solution was added an acyl isocyanate (10.5 mmol) at 0 °C under argon with stirring. The reaction mixture was stirred for 2 h at room temperature under argon, evaporated to dryness, and recrystallized from benzene for **2p** and from acetonitrile for **2t** and **2e**. Thiol **2** had the following properties (the melting points and NH proton chemical shifts are listed in Table 3).

2p: NMR ($CDCl_3$) δ=1.44 (1H, t, J=7.6 Hz, SH), 2.72 (2H, m, $HNCH_2$), 3.54 (2H, m, CH_2SH), 7.2—8.3 (5H, m, ArH). Found: C, 53.78; H, 5.42; N, 12.44; S, 14.28%; M^+, 224. Calcd for $C_{10}H_{12}N_2O_2S$: C, 53.57; H, 5.39; N, 12.50; S, 14.27%; M, 224.

2t: NMR ($CDCl_3$) δ=1.44 (1H, t, J=8.6 Hz, SH), 2.44 (3H, s, $ArCH_3$), 2.74 (2H, m, $HNCH_2$), 3.56 (2H, m, CH_2SH), 7.2—8.1 (4H, m, ArH). Found: C, 55.28; H, 5.82; N, 11.82; S, 13.17%; M^+, 238. Calcd for $C_{11}H_{14}N_2O_2S$: C, 55.45; H, 5.92; N, 11.76; S, 13.43%; M, 238.

2e: NMR ($CDCl_3$) δ=1.26 (3H, t, J=7.5 Hz, CH_3), 1.45 (1H, t, J=8.0 Hz, SH), 2.5—2.9 (4H, m, $C\underline{H}_2NH$ and $C\underline{H}_2CH_3$), 3.56 (2H, m, $HSCH_2$), 7.1—8.1 (4H, m, ArH). Found: C, 57.24; H, 6.42; N, 11.16; S, 12.72%; M^+, 252. Calcd for $C_{12}H_{16}N_2O_2S$: C, 57.13; H, 6.39; N, 11.11; S, 12.69%; M, 252.

Preparation of Disulfides. Symmetrical disulfides **5** and **8** were easily obtained by treatment with O_2 in the presence of Et_3N in CH_3CN at room temperature; disulfide **5** was recrystallized from CH_3CN–DMF. Satisfactory spectral data were used to support the assignment of disulfides **5**—**10**. Unsymmetrical disulfides **6** and **9** were prepared as described before,[46] recrystallized from CH_3CN for **6**, and had the following properties (NMR spectra of **5** and **6** were measured in Me_2SO-d_6 at 34 °C and 0.040 M).

5: Mp 210 °C; R_f 0.67 (silica, Et_2O–$EtCO_2H$–MeOH, 30:3:1) or 0.70 (silica, Et_2O–$EtCO_2H$–MeOH–AcOEt, 60:6:1:1); NMR δ=2.89 (12H, s, $N(CH_3)_2$), 3.81 (4H, s, SCH_2CO), 6.5—7.5 (8H, m, ArH), 10.02 (2H, s, NH^α), 10.65 (2H, s, NH^β). Found: C, 52.09; H, 5.58; N, 16.67; S, 12.95%. Calcd for $C_{22}H_{28}N_6O_4S_2$: C, 52.37; H, 5.59; N, 16.66; S, 12.69%.

6p: Mp 187 °C; R_f 0.83 (silica, Et_2O–$EtCO_2H$–MeOH–AcOEt, 60:6:1:1); NMR δ=2.7—3.8 (4H, m, SCH_2CH_2N), 2.84 (6H, s, $N(CH_3)_2$), 3.72 (2H, s, SCH_2CO), 6.5—8.3 (8H, m, ArH), 8.88 (1H, t, J=6 Hz, NH^δ), 10.03 (1H, s, NH^α), 10.68 (2H, s, NH^β and NH^γ). Found: C, 52.89; H, 5.33; N, 14.60; S, 13.54%. Calcd for $C_{21}H_{25}N_5O_4S_2$: C, 53.04; H, 5.31; N, 14.73; S, 13.48%.

6t: Mp 192 °C; R_f 0.84 (silica, Et_2O–$EtCO_2H$–MeOH, 30:3:1); NMR δ=2.37 (3H, s, $ArCH_3$), 2.83 (6H, s, $N(CH_3)_2$), 2.6—3.8 (4H, m, SCH_2CH_2N), 3.72 (2H, s, SCH_2CO), 6.5—8.1 (8H, m, ArH), 8.89 (1H, t, J=6 Hz, NH^δ), 10.04 (1H, s, NH^α), 10.60 (1H, s, NH^β), 10.67 (1H, s, NH^γ). Found: C, 54.15; H, 5.58; N, 14.47; S, 13.07%. Calcd for $C_{22}H_{27}N_5O_4S_2$: C, 53.97; H, 5.56; N, 14.30; S, 13.10%.

6e: Mp 195 °C; R_f 0.81 (silica, Et_2O–$EtCO_2H$–MeOH, 30:3:1); NMR δ=1.19 (3H, t, J=7.5 Hz, $ArCH_2C\underline{H}_3$), 2.6—3.8 (6H, m, SCH_2CH_2N and $ArCH_2$), 2.82 (6H, s, $N(CH_3)_2$), 3.71 (2H, s, SCH_2CO), 6.5—8.1 (8H, m, ArH), 8.89 (1H, t, J=6 Hz, NH^δ), 10.03 (1H, s, NH^α), 10.60 (1H, s, NH^β), 10.64 (1H, s, NH^γ). Found: C, 54.68; H, 5.77; N, 13.83; S, 12.53%. Calcd for $C_{23}H_{29}N_5O_4S_2$: C, 54.86; H, 5.81; N, 13.91; S, 12.71%.

*2-(Dimethylamino)ethyl Phenyl Disulfide (**9p**)*: Oil; R_f 0.38 (silica, n-C_6H_{14}–EtOH, 17:2); NMR ($CDCl_3$) δ=2.20 (6H, s, $N(CH_3)_2$), 2.4—3.0 (4H, m, SCH_2CH_2N), 6.8—7.8 (5H, m, ArH); M^+, 213.

2-(Dimethylamino)ethyl p-*Tolyl Disulfide (**9t**)*: Oil; R_f 0.39 (silica, n-C_6H_{14}–EtOH, 17:2); NMR ($CDCl_3$) δ=2.18 (6H, s, $N(CH_3)_2$), 2.28 (3H, s, $ArCH_3$), 2.3—2.9 (4H, m, SCH_2CH_2N), 6.8—7.8 (4H, m, ArH); M^+, 227.

*Preparation of Sulfide (**11**).* To a mixture of iodoacetamide (10 mmol) and Et_3N (11 mmol) in 100 ml of CH_3CN was added **1** (10 mmol) with stirring at 0 °C under argon. Stirring was continued for 1 h at room temperature. The reaction mixture was evaporated to dryness, washed with water, dried under reduced pressure over phosphorus pentaoxide, recrystallized from CH_3CN to give a pale yellow crystal, and had the following properties (NMR spectra were measured in Me_2SO-d_6 at 34 °C and 0.040 M): mp 195 °C; R_f 0.40 (silica, Et_2O–$EtCO_2H$–MeOH, 30:3:1) or 0.37 (silica, Et_2O–$EtCO_2H$–MeOH–AcOEt, 60:6:1:1); NMR δ=2.84 (6H, s, $N(CH_3)_2$), 3.24 (2H, s, $S\underline{C}H_2CONH$), 3.45 (2H, s, $SC\underline{H}_2CONH_2$), 6.5—7.5 (6H, m, $CONH_2$ and ArH), 10.06 (1H, s, NH^α), 10.58 (1H, s, NH^β). Found: C, 50.41; H, 5.87; N, 18.31; S, 10.55%. Calcd for $C_{13}H_{18}N_4O_3S$: C, 50.31; H, 5.85; N, 18.06; S, 10.31%.

Oxidation of a Pair of Thiols. A mixture of **1** (0.50 mmol) and **2** (0.50 mmol) [or of **3** (0.50 mmol) and **4** (0.50 mmol)] in 12.5 ml of 80% (v/v) CH_3CN–20% H_2O was stirred vigorously under oxygen for 15 min in a well-stirred water bath (for 20 to 70 °C) or methanol bath (for −10 and 0 °C) which was thermostated to ±0.1 °C for 20 to 50 °C and to ±0.5 °C for −10, 0, and 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. When the oxidation was completed, the reaction mixture was evaporated to dryness. In the case of the oxidation of system A, the yields of **5** and **6** were determined by the use of their absorption at 310 nm (ε=20300 for **5**, 10300 for **6p**, 10500 for **6t**, and 10400 for **6e** in 90% (v/v) THF–10% H_2O) after separation by TLC [2 mm×1.2 cm×5 cm E. Merck precoated silica gel plates (60 F_{254}), Et_2O–$EtCO_2H$–MeOH (30:3:1) for systems A_t and A_e or Et_2O–$EtCO_2H$–MeOH–AcOEt (60:6:1:1) for system A_p] followed by elution with 90% (v/v) THF–10% H_2O.[12] The yield shown in Table 1 represents the mean value of four or more measurements, and was reproducible to <3% average deviation from the mean. With oxidation of system N, the yields of **8** and **9** were determined gravimetrically after

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** 1 mmHg≈133.322 Pa.

separation by preparative TLC [1 mm×20 cm×20 cm silica gel plates (E. Merck 60 PF_{254}), n-C_6H_{14}–EtOH (17:2)] followed by elution with CH_2Cl_2 for **8** and with CH_3CN for **9**.

Thiol-Disulfide Exchange Reaction. A mixture of **2** (0.50 mmol) and **5** (0.25 mmol) was stirred in 12.5 ml of 80% (v/v) CH_3CN–20% H_2O vigorously under argon for 15 min in a well-stirred water bath or methanol bath which was thermostated as in the oxidation. To this mixture was added Et_3N (0.05 mmol), and vigorous stirring was continued for the same time as with the corresponding oxidation under argon. To this mixture were added iodoacetamide (0.55 mmol) and Et_3N (0.55 mmol). After vigorous stirring for 10 min at 35 and 70 °C or for 20 min at 0 °C, the resulting mixture was evaporated to dryness. The yields of **5**, **6**, and **11** (ε=10300 at 310 nm in 90% (v/v) THF–10% H_2O) were determined as described with the oxidation. The yield of the corresponding sulfide derived from **2** could not be determined by the method[12] because of its small molar extinction coefficient (ε=*ca.* 30 in 90% (v/v) THF–10% H_2O at 310 nm). Similarly, the exchange reaction between **1** and **7** was carried out. The yield shown in Table 2 represents the mean value of four or more measurements, and was reproducible to <3% average deviation from the mean.

Disulfide-Disulfide Exchange Reaction. A mixture of **5** (0.25 mmol) and **7t** (0.25 mmol) was stirred vigorously in 12.5 ml of 80% (v/v) CH_3CN–20% H_2O for 15 min in a well-stirred water bath held at 35.0 °C controlled to ±0.1 °C. To this mixture was added Et_3N (0.025 mmol), and vigorous stirring was continued for 3 h. The resulting reaction mixture was evaporated to dryness, and the yields of **5** and **6t** were determined as described above.

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References

1) H. O. Smith, *Science*, **205**, 455 (1979).

2) T. Lindahl, *Nature*, **290**, 363 (1981); M. Ehrlich and R. Y. -H. Wang, *Science*, **212**, 1350 (1981).

3) W. K. Paik and S. Kim, "Protein Methylation," Wiley, New York (1980).

4) F. Hirata and J. Axelrod, *Science*, **209**, 1082 (1980).

5) G. L. Cantoni, *Ann. Rev. Biochem.*, **44**, 435 (1975).

6) W. Arber, *Angew. Chem., Int. Ed. Engl.*, **17**, 73 (1978).

7) A. Razin and A. D. Riggs, *Science*, **210**, 604 (1980) and the references cited therein.

8) B. Griffin, *Nature*, **263**, 188 (1976); M. J. Clemens, *ibid.*, **279**, 673 (1979).

9) G. L. Hazelbauer, *Nature*, **279**, 18 (1979); M. S. Springer, M. F. Goy, and J. Adler, *ibid.*, **280**, 279 (1979); A. L. DeFranco and D. E. Koshland, Jr., *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 2429 (1980).

10) The symbols p, t, and e represent the compounds or systems to have the phenyl, p-tolyl, and p-ethylphenyl groups, respectively, as R^1 or R^2.

11) T. Endo, Y. Takeda, T. Orii, Y. Kaneko, and M. Kondo, *Chem. Lett.*, **1979**, 1455.

12) This method improved the accuracy of quantitative analysis of substances of low solubility: T. Endo, A. Kuwahara, H. Tasai, and T. Ishigami, *J. Chromatogr.*, **140**, 263 (1977).

13) Disulfide **7** could not be determined by this method[12] because of its very small molar extinction coefficient (ε) at wavelengths longer than 260 nm in 90% (v/v) tetrahydrofuran–10% water: the ε value for **7** was *ca.* 30 at 310 nm.

14) In oxidation of system N, the symmetrical disulfide used for the calculation of R was **8** derived from **4**, but not **10** from **3**, because the spot of **10** on TLC plates could not be detected in UV light.

15) Oxidation of a mixture of methanethiol and ethanethiol yielded the three disulfides in a 1:2:1 ratio: D. T. McAllan, T. V. Cullum, R. A. Dean, and F. A. Fidler, *J. Am. Chem. Soc.*, **73**, 3627 (1951).

16) T. Endo, A. Kuwahara, H. Tasai, T. Murata, M. Hashimoto, and T. Ishigami, *Nature*, **268**, 74 (1977).

17) a) P. C. Jocelyn, "Biochemistry of the SH group," Academic, London (1972), Chap. 5; b) L. Eldjarn and A. Pihl, *J. Am. Chem. Soc.*, **79**, 4589 (1957); c) A. Fava, A. Iliceto, and E. Camera, *ibid.*, **79**, 833 (1957); d) R. P. Szajewski and G. M. Whitesides, *ibid.*, **102**, 2011 (1980).

18) This nucleophilic substitution (Eq. 4) is known to proceed very rapidly, and extensively used in protein chemistry: A. Fontana and C. Toniolo, "Detection and Determination of Thiols," in "The Chemistry of the Thiol Group," ed by S. Patai, Wiley, London (1974), Part 1, Chap. 5.

19) If a single multimer species in equilibrium with monomer is assumed to be dimer, f values range from 1.0 to 2.0: M. Davies and D. K. Thomas, *J. Phys. Chem.*, **60**, 763 (1956).

20) J. H. Hildebrand, E. T. Ellefson, and C. W. Beebe, *J. Am. Chem. Soc.*, **39**, 2301 (1917).

21) Surface tension is reported to be 71.97 for water (25 °C) and 29.3 for CH_3CN (20 °C): "Lange's Handbook of Chemistry," 11th ed, ed by J. A. Dean, McGraw-Hill, New York (1973), Section 10.

22) Similar, but less dramatic, variations in temperature-selectivity profiles were observed in oxidation of system A_a: a plot of R in 80% (v/v) CH_3CN–20% H_2O against temperature showed a peak of maximum selectivity (R=21) at 35 °C for R^1=i-C_5H_{11}, whereas for R^1=n-C_5H_{11} the R values altered progressively over a wide range (0.13 to 2.3).[16]

23) We have recently prepared a great variety of solid 1:1 complexes between a pair of acylurea bond-containing compounds (analogs of **1** and **2**) each with the p-(dimethylamino)phenyl or p-nitrophenyl group as one of the two substituents. X-Ray crystallographic studies of the two complexes and **1** have indicated that (1) they have the same pattern of hydrogen bonding as in Fig. 4 and (2) the packing modes of the four molecules in the unit cells of the complexes and of **1** are analogous to the association schemes of heterotetramer **18** and homotetramer **15**, respectively (Y. Ohashi, A. Uchida, and Y. Sasada, private communication). These observations encourage our view that there exist tetramers **15**—**19** in solution (Fig. 5).

24) Among many possibilities, five tetramers were chosen so that (1) the ratio of **1** to **2** in heterotetramers might be 1:1 and (2) the stabilization of tetramers might become strongest.[11]

25) Tetramer formation is shown for methanol in the gas (W. Weltner, Jr., and K. S. Pitzer, *J. Am. Chem. Soc.*, **73**, 2606 (1951)); the important multimer species of both methanethiol and ethanethiol in argon matrices at low concentrations at 20 K is the open chain dimer and cyclic tetramer, the latter being particularly stable for ethanethiol (A. J. Barnes, H. E. Hallam, and J. D. R. Howells, *J. Chem. Soc., Faraday Trans. 2*, **68**, 737 (1972)). For the other examples, see Ref. 11.

26) M. R. Crampton, "Acidity and Hydrogen-Bonding," in "The Chemistry of the Thiol Group," ed by S. Patai, Wiley, London (1974), Part 1, Chap. 8.

27) T. Endo, Y. Takeda, H. Kamada, S. Kayama, and H. Tasai, *Chem. Lett.*, **1980**, 417.

28) N. C. Seeman, J. M. Rosenberg, and A. Rich, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 804 (1976).

29) E. Hutchinson and K. Shinoda, "An Outline of the Solvent Properties of Surfactant Solutions," in "Solvent Properties of Surfactant Solutions," ed by K. Shinoda, Marcel Dekker, New York (1967), Chap. 1.

30) E. H. Cordes and C. Gitler, *Prog. Biorg. Chem.*, **2**, 1 (1973); J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems," Academic, New York (1975), Chap. 2.

31) P. Rys, *Angew. Chem., Int. Ed. Engl.*, **16**, 807 (1977).

32) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York (1969), Chap. 8; C. Tanford, *Science*, **200**, 1012 (1978); H. A. Scheraga, *Acc. Chem. Res.*, **12**, 7 (1979); A. B. Naim, "Hydrophobic Interactions," Plenum, New York (1980).

33) The π value, an excellent parameter of hydrophobicity, increases in the order $CH_3 < C_2H_5 < i\text{-}C_3H_7 < n\text{-}C_3H_7 < i\text{-}C_4H_9 < n\text{-}C_4H_9 < n\text{-}C_5H_{11}$: C. Hansch, A. R. Steward, J. Iwasa, and E. W. Deutsch, *Mol. Pharmacol.*, **1**, 205 (1965).

34) A linear relationship is known to exist between "accessible surface area" (B. Lee and F. M. Richards, *J. Mol. Biol.*, **55**, 379 (1971)) for the amino acid side chains and hydrophobicity, represented by free energy change for the transfer of amino acid side chains from 100% organic solvents to water at 25 °C (C. Chothia, *Nature*, **248**, 338 (1974)). The "relative surface areas," estimated by covering CPK atomic models, were shown to be closely correlated with free energies of transfer of saturated hydrocarbon groupings from water to a nonpolar phase (S. M. J. Harris, T. Higuchi, and J. H. Rytting, *J. Phys. Chem.*, **77**, 2694 (1973)).

35) J. P. Guthrie, "Enzyme Models and Related Topics," in "Applications of Biochemical Systems in Organic Chemistry," ed by J. B. Jones, C. J. Sih, and D. Perlman, Wiley, New York (1976), Part II, Chap. III.

36) G. Némethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3401 (1962); see also S. J. Gill and I. Wadsö, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2955 (1976).

37) Similar phenomenon has been observed in the case of oxidation of system A_a ($R^1 = i\text{-}C_5H_{11}$).[16]

38) R. Lawaczeck and K. G. Wagner, *Biopolymers*, **13**, 2003 (1974).

39) R. D. Cramer III, *J. Am. Chem. Soc.*, **99**, 5408 (1977).

40) a) J. H. Hildebrand, *J. Phys. Chem.*, **72**, 1841 (1968); see response by G. Némethy, H. A. Scheraga, and W. Kauzmann, *ibid.*, **72**, 1842 (1968); b) J. H. Hildebrand, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 194 (1979); see response by C. Tanford, *ibid.*, **76**, 4175 (1979).

41) T. Endo, Y. Takeda, T. Orii, T. Murata, M. Sakai, N. Nakagawa, and K. Nikki, *Chem. Lett.*, **1980**, 1291.

42) Indeed, CH···N interaction between an alkyl group (R^1) in **2a** and the amino group [$N(CH_3)_2$] in **1** was observed to be specific for R^1, namely, to depend largely on whether R^1 was the isopentyl or pentyl group, through alterations in the NH proton chemical shifts.[41]

43) Specific weak interactions which would seem to operate between the recognition sites and between the recognition site and reaction site in **1** and **2** are CH···N interaction between the R^1 and $N(CH_3)_2$ groups, CH···CH interaction between the R^1 and methyl (and/or phenyl) groups and between the two R^1 groups, and CH···SH interaction between the R^1 and SH groups (Figs. 4 and 5).

44) M. H. Abraham, *J. Am. Chem. Soc.*, **102**, 5910 (1980). See also K. Shinoda and M. Fujihira, *Bull. Chem. Soc. Jpn.*, **41**, 2612 (1968) and K. Shinoda, *J. Phys. Chem.*, **81**, 1300 (1977).

45) T. Endo, K. Oda, and T. Mukaiyama, *Chem. Lett.*, **1974**, 443.

46) T. Endo, H. Tasai, and T. Ishigami, *Chem. Lett.*, **1975**, 813.