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A Basic Study of the Amino Acid Residue in Protein. The Molecular Interaction Responsible for the Differentiation of Hydrocarbon Residues

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A pair of primary amines was competitively acylated with acid anhydride in water–dioxane mixed solvents. Based on the product distribution, the mode of differentiation of hydrocarbon residues in the competing substrates with a reagent was studied. In solvents with a low water content, where both reactants and products were completely soluble, no appreciable differentiation of the alkyl-chain length of alkylamines occurred. On the other hand, the differentiation of a phenyl group from an alkyl group occurred. From the dependency of the product distribution on the water content in reaction media, an attractive interaction induced by water was postulated between alkyl and phenyl groups, but no such interaction was observed between alkyl and alkyl groups. The differences in functions of the phenyl and alkyl groups in the differentiation process are discussed. In solvents with a very high water content, the acylation proceeded under heterogeneous conditions, since acid anhydrides became insoluble and provided an organic phase in the reaction mixture. In this case, the differentiation was controlled by the hydrophobicity of hydrocarbon residues of amines indexed by the partition coefficients of amines between the aqueous phase and the organic phase.

Among the various molecular interactions affecting the molecular recognition,[1–5] the interaction between hydrocarbon residues of reactant molecules is one of the essential factors in understanding the various phenomena found in the biological and organic reactions in either an aqueous or nonaqueous environment.

A systematic investigation of the competitive acylation of primary amines with an acid anhydride (Eq. 1) was carried out in order to characterize the interactions between such medium-size hydrocarbon residues as found in common amino acids in proteins.

As was briefly reported in a previous communication,[6] this reaction system had the following advantages: 1) the reaction readily proceeded in a quantitative manner in kinds of reaction media, including an aqueous one, and gave kinetically controlled products; 2) the reaction products were stable and could readily be analyzed by means of quantitative gas chromatography and; 3) the substrates and reagents substituted with many different hydrocarbon groups were readily available.

The contributions of the hydrocarbon residues to the molecular recognition in the course of the reaction (the differentiation process) were evaluated by the use of logarithmic molar ratio of products (Eq. 2):

$$\left.\begin{matrix}R^1\text{-}NH_2\\ R_2\text{-}NH_2\end{matrix}\right\} + (R^0\text{-}CO)_2O \longrightarrow \left\{\begin{matrix}R^1\text{-}NHCO\text{-}R^0\\ R^2\text{-}NHCO\text{-}R^0\end{matrix}\right. \qquad (1)$$

$$\ln r = \ln(R^1\text{-}NHCO\text{-}R^0/R^2\text{-}NHCO\text{-}R^0), \qquad (2)$$

where R^1, R^2–, and R^0– are the hydrocarbon residues.

Experimental

Instruments. The ^{1}H-NMR and IR spectra were taken with a JEOL FX-100 spectrometer and a Shimadzu IR 27 G spectrometer respectively. The analytical GLC was carried out with a Shimadzu GC 6A gas chromatograph equipped with a Shimadzu Chromatopack C-RIA, using a 3 m-5 mm o.d. glass column packed with 2% Silicon OV-17 on Chromosorb W (OV-17) at the stated temperature. The preparative GLC was carried out with a Shimadzu 3 A instrument, using a 3 m-6 mm o.d. stainless column packed with OV-17.

Materials. All the chemical except those noted below were obtained from commercial sources and were used without further purification. The phenylacetic anhydride was prepared from the corresponding acid chloride and sodium salt of the acid by the conventional method. The needle crystals were obtained by recrystallization from ether–hexane (mp 71 °C, lit, 72 °C). The 2-phenylbutyric anhydride was prepared from the corresponding acid by refluxing with a large excess of acetic anhydride, followed by the removal of the acetic acid released by distillation. The *N*-acetyl-L-isoleucine *N*-hydroxysuccinimide ester was prepared from *N*-acetyl-L-isoleucine and *N*-hydroxysuccinimide by treatment with dicyclohexylcarbodiimide according to a conventional method; it was then purified by recrystallization from isopropyl alcohol. The authentic samples for the identification of reaction products were prepared from appropriate acid chlorides and amines by a conventional method, and were purified by preparative GLC. The NMR and IR spectra and the elemental analyses of the samples were consistent with the desired structures of the samples.

Reaction Procedure of Competitive Acylation. When the concentration of an acylating reagent was 50 mM (1 mM= 10^{-3} mol dm^{-3}), the competitive acylation was carried out by the following procedure. In a flask, a 50-μl portion of a stock solution of each amine (2 M dioxane solution) was dissolved in 0.8 ml of the solvent. A 100-μl portion of a solution of anhydride (0.5 M dioxane solution) was then added, all at once, with vigorous stirring at room temperature, after which the mixture was allowed to stand for 1 h. After a 100-μl portion of a stock solution of an internal standard (0.25 M dioxane solution), which will be described is detail, in the following section, had been added for GLC analysis, the volume of the reaction mixture was made up to 5 ml with dioxane. GLC was used for the quantitative analysis of the products of the reaction solution. When the concentration of the acylating reagent was 6.25 mM, 25-μl of each amine solution, 50-μl of the acid anhydride solution, 3.9 ml of the solvent, and 50-μl of the internal standard solution were employed instead.

Quantitative Analysis of Reaction Products. The reaction mixture prepared as has been described in the previous section was subjected to GLC analysis. The amount of the product was determined by the internal standard method. The analyses of the 2-phenylbutyramides and phenylacetamides were carried out in a column with a temperature elevated from 150 °C to 250 °C by 10 °C/min; methyl stearate was used as the internal standard. The 4-methylpentanamides were analyzed under such GLC conditions that the column temperature was elevated from 100 °C to 260 °C by 10 °C/min, with methyl palmitate as the internal standard. The *N*-acetyl-L-isoleucinamides, isobutyramides, and isovaleramides were analyzed under such GLC conditions that the column temperature was elevated from 100 °C to 250 °C by 10 °C/min, with acetanilide as the internal standard.

Solubility of Acid Anhydrides in Aqueous Media. The solubility of an acylating reagent in water–dioxane mixed solvents was determined as follows. A 100-μl portion of a acylating reagent (0.5 M dioxane solution) was vigorously stirred into a water–dioxane mixture (0.9 ml). The resultant mixture was centrifuged at 2000 min^{-1} for 20 min to separate any undissolved acylating reagent from the solvent phase. A 50-μl portion of butylamine (2 M dioxane solution) was added to the 100-μl portion of supernatant obtained under vigorous stirring, and the mixture was allowed to stand for 1 h to complete the reaction. The quantitative GLC analysis of the *N*-butyl amides was carried out by following the procedure described in the above section.

Results

The Competitive Reaction Conditions. The competitive acylation was carried out at room temperature by using an equimolar mixture of two primary amines as substrates. The ln *r* value depends not only on the interactions between substrates and the reagent in the differentiation process, but also on the changes in the relative concentrations of the two substrates during the reaction. Thus, the reaction should be carried out in the presence of so large an excess of the substrates relative to the reagent as to eliminate the effects of the concentration changes in the substrates. To establish the appropriate reaction conditions, the ln *r* values were determined under a variety of molar ratios of substrates relative to reagent ($[R^1\text{–}NH_2+R^2\text{–}NH_2]/[(R^0\text{–}CO)_2O]$, as is shown in Fig. 1. The ln *r* value became constant when the relative molar ratio of substrates to a reagent was over four. Therefore, four molar equivalents of substrates were treated with one molar equivalent of a reagent in the following experiments. With this ratio of substrates to a reagent, it was also confirmed that the ln *r* value was not affected by the carboxylic acids liberated during the reaction: the same ln *r* values were obtained in the presence and in the absence of triethyl amine, an acid quencher.

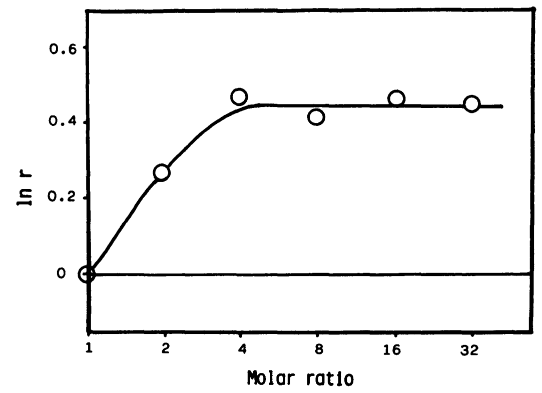
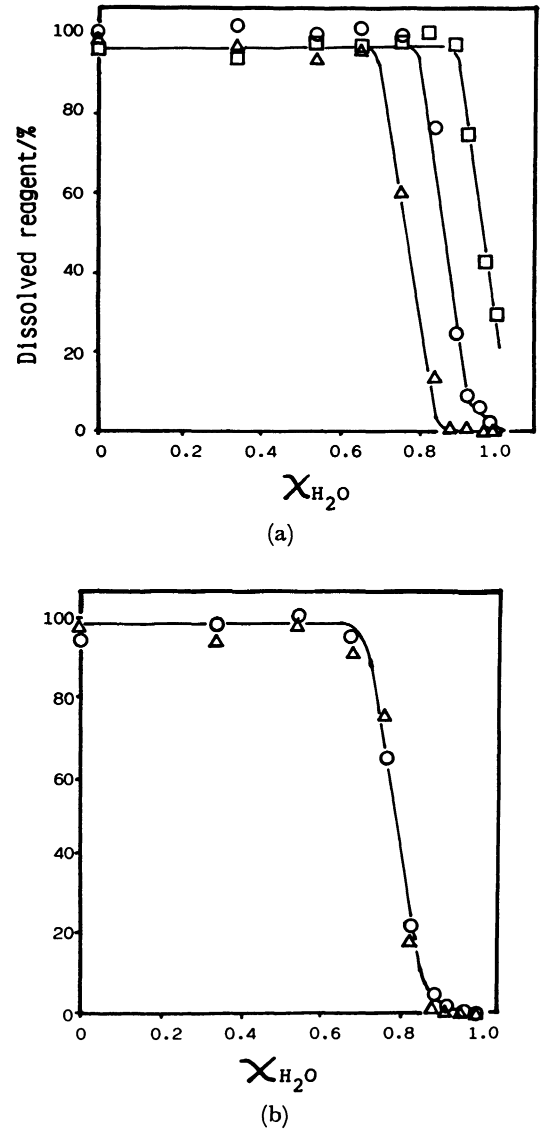


Fig. 1. The dependence of the ln *r* value on the molar ratio of substrates to a reagent ($[R^1\text{–}NH_2+R^2\text{–}NH_2]/[(R^0\text{–}CO)_2O]$).
Isovaleric anhydride, hexylamine, and propylamine were employed as $(R^0\text{–}CO)_2O$, $R^1\text{–}NH_2$, and $R^2\text{–}NH_2$, respectively. The reaction was carried out in water–dioxane mixtures of $\chi_{H_2O}=0.88$. The concentration of isovaleric anhydride was 6.25 mM.

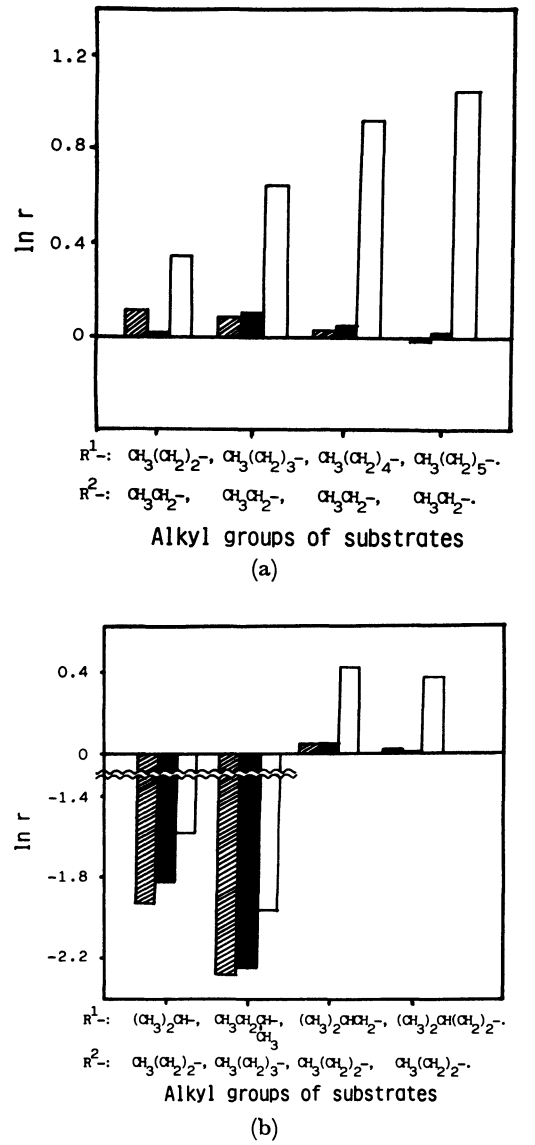


(a)

(b)

Fig. 2. The dependence of the amount of dissolved reagent (% of applied reagent) on the mole fraction of water (χ_{H_2O}) in water–dioxane mixtures.
In (a), 4-methylpentanoic anhydride (△), isovaleric anhydride (○), and isobutyric anhydride (□) were employed. Their concentration was 50 mM if all dissolved. In(b), 2-phenylbutyric anhydride (○) and phenylacetic anhydride (△) were employed. Their concentration was 12.5 mM if all dissolved.

Benzene, dioxane, and water–dioxane mixtures were employed as solvents. Figure 2 shows the relationship between the solubility of a reagent and the mole fraction of water (χ_{H_2O}) in the water–dioxane mixture. As may be found in the figure, the solubility of an acid anhydride suddenly decreased above a certain value of χ_{H_2O}. When an acid anhydride was added to a solution with a value above this critical χ_{H_2O}, the formation of a distinct organic phase was observed.



(a)

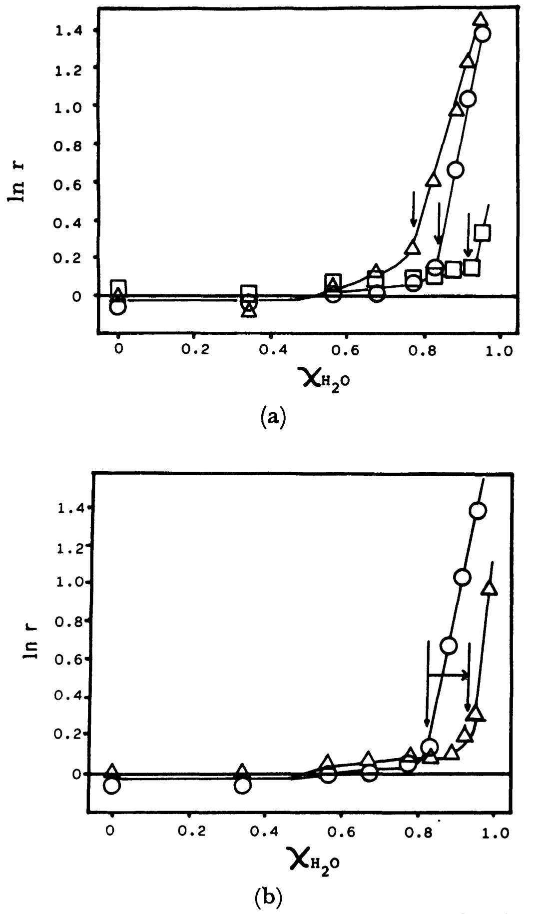
(b)

Fig. 3. (a) and (b). The competitive acylation of a pair of alkylamine with isovaleric anhydride.
The reaction was carried out in benzene (▨), dioxane (■), or water–dioxane mixtures at $\chi_{H_2O}=0.88$ (□). The concentration of a reagent was 50 mM.

The Competition of Alkylamines with Different Chain Lengths. A pair of alkylamine were competitively acylated with isovaleric anhydride in benzene, dioxane, or water–dioxane mixtures ($\chi_{H_2O}=0.88$). The results are shown in Fig. 3.

In dioxane or benzene, the ln *r* values were found to be substantially zero in every competition between unbranched alkylamines (Fig. 3(a)), and in the competition of a *β*- or *γ*-branched amine with an unbranched one (Fig. 3(b)). In the competition of an *α*-branched alkylamine, such as isopropylamine and *s*-butylamine, with the unbranched amines, large negative ln *r* values were obtained in every solvent; the formation of amides from *α*-branched amines was significantly suppressed, as is shown in Fig. 3(b).

In aqueous media (water–dioxane), positive ln *r* values were obtained except in the competition of an *α*-branched amine with an unbranched one (Fig. 3(a) and (b)). The ln *r* values obtained in the competitions of unbranched amines in water–dioxane mixtures are shown in Table 1. Thus, the reagent distinguished the longer alkylamine from the shorter one and preferentially gave the amide with the longer alkylamine.



(a)

(b)

Fig. 4. The relationship between the mole fraction of water in water–dioxane mixtures (χ_{H_2O}) and the ln *r* value of the competitive acylation of hexylamine (R^1–NH_2) and propylamine (R^2–NH_2).
In (a), 4-methylpentanoic anhydride (△), isovaleric anhydride (○), and isobutyric anhydride (□) were employed. Their concentration was 50 mM. In (b), isovaleric anhydride was employed. Its concentration was 50 mM (○) or 6.25 mM (△).

In order to ascertain the mode of the differentiation in an aqueous solvent, the competition of hexylamine with propylamine was carried out in water–dioxane mixtures with a variety of χ_{H_2O} values. The profile of the resulting plots was apparently composed of two regions: Phase I and Phase II. For example, the plot (○) in Fig. 4(a) was composed of Phase I ($\chi_{H_2O}<0.83$) and Phase II ($\chi_{H_2O}>0.83$). In Phase I, no effective differentiation of the alkyl-chain length was observed, while considerable differentiation was observed in Phase II, and ln *r* remarkably increased with the increase in χ_{H_2O}.

In the acylation carried out in solvents with $\chi_{H_2O}>0.9$, it was observed that the reaction mixture became slightly turbid just after the addition of a reagent and that the turbidity disappeared instantaneously. The same ln *r* values were obtained in the competitive acyla-

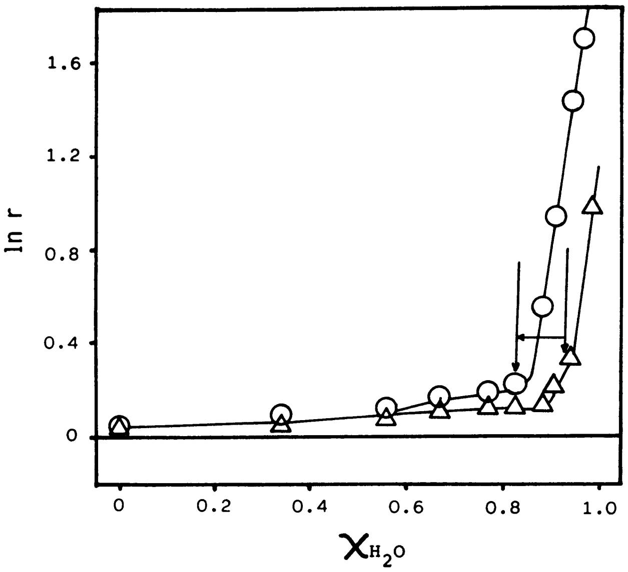


Fig. 5. The relationship between the mole fraction of water in water–dioxane mixtures (χ_{H_2O}) and the ln *r* value of the competitive acylation in the presence (○) or the absence (△) of CTAB.
Isovaleric anhydride, hexylamine, and propylamine were employed as $(R^0\text{-}CO)_2O$, $R^1\text{-}NH_2$, and $R^2\text{-}NH_2$, respectively. The concentration of a reagent was 6.25 mM.

tion carried out in two different manners: one was the reaction carried out by the addition of an acid anhydride to amine mixtures homogeneously dissolved in solvents the standard procedure is described in the experimental section, while the other is the reaction carried out by the addition of amine mixtures to suspensions of reagents previously prepared in solvents with large χ_{H_2O} values. These facts strongly suggested that the occurrence of two regions in the plots of ln *r vs.* χ_{H_2O} is related to the solubility of acid anhydrides in water–dioxane mixed solvents. A comparison of Figs. 4(a) and 2(a) clearly shows that the reaction took place under homogeneous conditions in Phase I, while in Phase II it took place under heterogeneous conditions. Accordingly, it can well be understood that the boundary between Phase I and Phase II shifted to a larger χ_{H_2O} value, from 0.82 to 0.9, in the eight-fold dilution of the reagent (Fig. 4(b)).

When cetyl trimethylammonium bromide (CTAB) was added to the reaction system, no apparent phase separation of the reagent occurred. However, Phase II appeared even in the presence of CTAB, as is shown in Fig. 5. The boundary between Phases I and II shifted to a smaller χ_{H_2O} value. Thus, the reagent incorporated into micelles provided by CTAB functioned much like the organic phases produced only by the aggregation of reagents.

When a water-soluble reagent such as the *N*-acetyl-L-isoleucine *N*-hydroxysuccinimide ester was used, Phase II did not appear. In this case, no effective differentiation of alkyl-chain length was observed, not even in solvents with a high χ_{H_2O} value as is shown in Table 1.

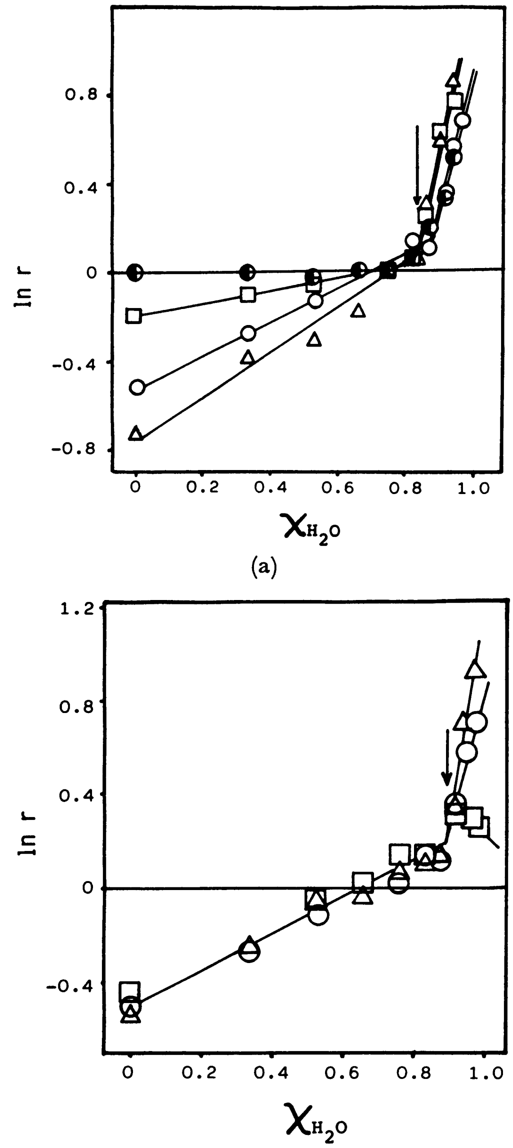


Fig. 6. The relationship between the mole fraction of water in water–dioxane mixtures (χ_{H_2O}) and the ln *r* value of the competitive acylation with isovaleric anhydride. In (a), benzylamine (△), phenethylamine (△), 3-phenylpropylamine (○), and pentylamine (◐) were employed as $R^1\text{-}NH_2$. Butylamine was employed as $R^2\text{-}NH_2$ in every case. The concentration of a reagent was 50 mM. In (b), phenethylamine was employed as $R^1\text{-}NH_2$. As $R^2\text{-}NH_2$, propylamine (△), butylamine (○), and hexylamine (□) were employed. The concentration of a reagent was 50 mM.

The Competition of an Alkylamine and a Phenylalkylamine. Figure 6(a) shows the results of the competitive acylation between a phenylalkylamine and an unbranched alkylamine with isovaleric acid anhydride (R^0=$-CH_2CH(CH_3)_2$). Benzylamine, phenethylamine, or 3-phenylpropylamine was employed as $R^1\text{-}NH_2$, and butylamine was employed as $R^2\text{-}NH_2$. The profile of the plots is composed of two phases: Phase I ($\chi_{H_2O}<0.83$) and Phase II ($\chi_{H_2O}>0.83$). In Phase II, the ln *r* values increased remarkably with the increase in χ_{H_2O}. In Phase I, the ln *r* value increased from negative to

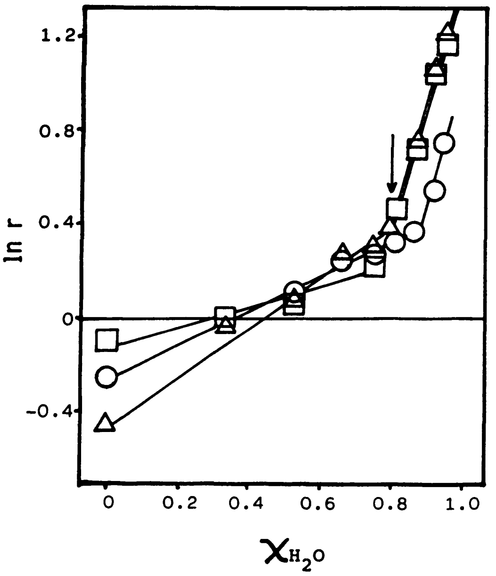


Fig. 7. The relationship between the mole fraction of water in water–dioxane mixtures (χ_{H_2O}) and the ln *r* value of the competitive acylation with isovaleric anhydride. Phenethylamine and butylamine were employed as R^1–NH_2 and R^2–NH_2, respectively. As a reagent, butyric anhydride (○), 2-phenylbutyric anhydride (△), and phenylacetic anhydride (□) were employed. The concentration of a reagent was 50 mM.

positive with the increase in χ_{H_2O}. This increase in ln *r* was more pronounced when the phenyl group was substituted near the amino group.

Figure 6(b) shows the ln *r vs.* χ_{H_2O} plots obtained by the competition of phenethylamine with propylamine, butylamine, or hexylamine. In Phase I, all the plots overlapped, even though the chain lengths of the alkylamines were different from one another. In Phase II, the slope of each plot was different. In the case of hexylamine, the ln *r* value decreased with the increase in χ_{H_2O} ((□) plot).

Figure 7 shows the results of the competitive acylation between phenethylamine and butylamine with several different types of reagents. In this case, the slopes of the plots in Phase I changed significantly with the change in the hydrocarbon residues of the reagents, whereas those of Phase II were similar to one another.

Figure 8 shows the results of the competitive acylation between amines with different pK_a's. The ln *r* value changed with χ_{H_2O} in Phase I only when benzylamine competed with others (the (○) and (□) plots). No change in the ln *r* value with a change in χ_{H_2O} was observed in any other combinations of amines (the (□) and (◧) plot). Thus, the changes in the ln *r* value with χ_{H_2O} in Phase I in the competition of phenylalkylamines with alkylamines are caused not by the difference in the pK_a's of the amines, but by the phenyl groups in the reactants.

Figure 9 shows the results of the competitive acylation in methanol–dioxane or acetonitrile–dioxane mixed solvents. At any composition of these solvents, the reaction proceeded under homogeneous conditions which corresponded to Phase I in water–dioxane solvents. As is shown by the (○) and (△) plots, the changes of

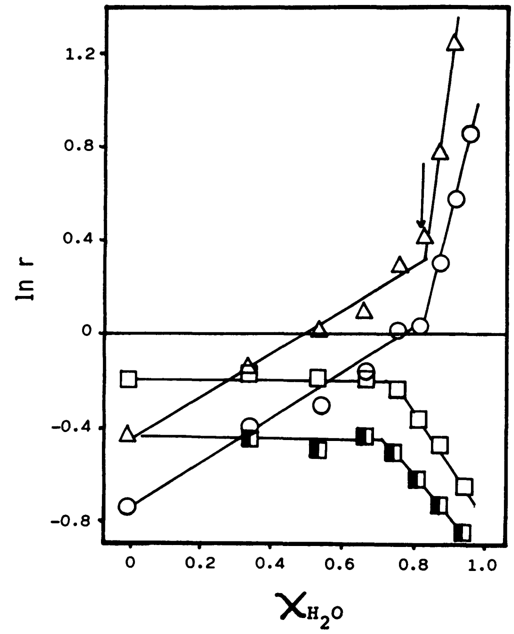


Fig. 8. The relationship between the mole fraction of water in water–dioxane mixtures (χ_{H_2O}) and the ln *r* value of the competitive acylation with isovaleric anhydride. The following combinations of R^1–NH_2 and R^2–NH_2 were examined: benzylamine and butyl-2-amine (○), benzylamine and 2-methoxyethylamine (△), 2-methoxyethylamine and butylamine (□), and 2-aminoethanol and butylamine (◧). The pK_a values of butylamine, benzylamine, 2-methoxyethylamine, and 2-aminoethanol were 10.64, 9.35, 9.28, and 9.5, respectively. The concentration of a reagent was 50 mM.

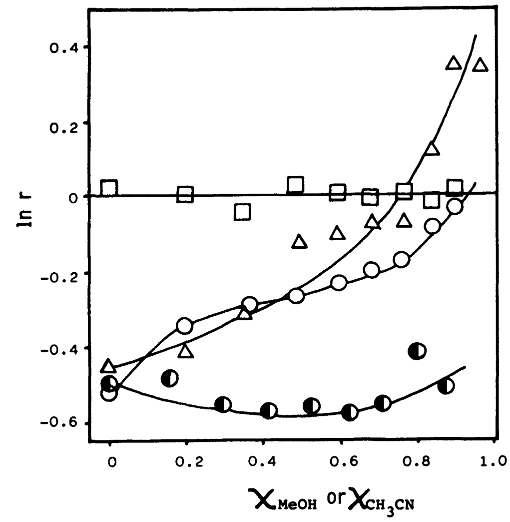


Fig. 9. The relationship between the mole fraction of methanol or acetonitrile in methanol–dioxane or acetonitrile–dioxane mixtures (χ_{MeOH} or χ_{CH_3CN}) and the ln *r* value.

The following combination of R^1–NH_2, R^2–NH_2, and $(R^0–CO)_2O$ were examined: phenethylamine, butylamine, and isovaleric anhydride in methanol–dioxane (○) and in acetonitrile–dioxane (◐), phenethylamine, butylamine, and 2-phenylbutyric anhydride in methanol–dioxane (△), hexylamine, butylamine, and isovaleric anhydride in methanol–dioxane (□). The concentration of a reagent was 50 mM.

the ln r value in Phase I were induced by either water or methanol, but no appreciable change in the ln r value was observed in acetonitrile–dioxane mixed solvents.

Discussion

In the acylation of a primary amine with an acid anhydride or an active ester, the rate-determining step is the nucleophilic attack by an amino group on a carbonyl carbon of the reagent.[7] When an excess amount of an amine ($\mathrm{p}K_a > 9$) is acylated by a reagent carrying a good leaving group such as an acid anhydride, the rate of acylation becomes insensitive to a small change in the nucleophilicity of an amino group, indexed by its $\mathrm{p}K_a$ value.[8] From the examination of competing reaction conditions, it was shown that the ln r value was not affected by the relative ratio of competing substrates during the reaction if the initial molar ratio of substrates to a reagent, *i.e.*, $[R^1\text{–}NH_2 + R^2\text{–}NH_2]/[(R^0\text{–}CO)_2O]$, was over four (Fig. 1). Under the experimental conditions employed in the study, the ln r value is expected to represent the degree of the differentiation of substrate molecules by a reagent.

In aprotic solvents, such as dioxane and benzene, the reagent molecule could distinguish only an α-branched substrate from an unbranched one, while it could distinguish neither the difference in the alkyl-chain length of unbranched substrates nor the β- and γ-branched substrates from an unbranched one (Fig. 3). These facts show that the differentiation in a nonaqueous system was controlled by the bulkiness of the alkyl groups near the reaction center.[4]

In Phase I of water–dioxane mixed solvents, the reaction proceeded under homogeneous conditions, where the differentiation of the substrates by the reagent must be controlled by molecular interactions. As may be found in Fig. 4(a) and (b), no effective differentiation of the chain length of alkyl groups took place, even in solvents with high χ_{H_2O} value, as long as the reaction took place in Phase I. Thus, it was shown that there is no characteristic interaction contributing to the differentiation of the chain length of alkyl groups in Phase I. The absence of interaction between alkyl groups in the reagent and substrate is also supported by the fact that the slope of plots in phase I in Fig. 6(b) were not affected by the alkyl-chain length of R^2–NH_2.

However, the ln r value increased with the increase in χ_{H_2O} from a negative value to a positive one in the competition of a phenylalkylamine with an alkylamine in Phase I. As may be found in Fig. 6(a), the slopes of the plots depended on the structural features of the phenylalkylamines. As may be found in Fig. 6(b), the slope of the plot was determined by the sort of phenylalkylamine and was not changed by the change in the chain length of alkylamines. The results shown in Fig. 8 exculde the possibility that the slope of a plot depends on the difference in the $\mathrm{p}K_a$'s of the competing amines. These facts can be explained in terms of an attractive interaction between the phenyl group of a substrate and the alkyl group of a reagent (an alkyl-phenyl interaction) that was induced by the addition of water to the reaction media. Since this sort of alkyl-phenyl interaction is a molecular interaction functioning under homogeneous conditions, it can participate in the differentiation of chirality, as was reported in the previous communication.[6]

When phenylacetic anhydride is used in the competitive acylation between phenethylamine and butylamine, the slope of the ln r *vs.* χ_{H_2O} plot in Phase I will represent the difference between a phenyl-phenyl interaction (an interaction between phenyl groups of a substrate and a reagent) and an alkylphenyl interaction. The slope of the (□) plot in Fig. 7 showed a more gentle gradient than that of the (○) plot in Fig. 7 or than those of the plots in Fig. 6(b). The results show that a phenyl-phenyl interaction was induced as well as an alkyl-phenyl interaction by the addition of water to solvents. The former was a little stronger than, or comparable to, the latter.

Since the alkyl-phenyl interaction occurred not only in water, but also in methanol, which has strong intermolecular hydrogen bonds, the interaction must be solvophobic in character. No alkyl-phenyl interaction occurred in dioxane or acetonitrile ($\varepsilon = 37.5$)[11] whose dielectric constant is comparable to that of methanol ($\varepsilon = 32.6$)[11] (Fig. 9).

The alkyl chains of substrates were remarkably differentiated in Phase II (Fig. 4 and Table 1). These results are in clear contrast to the results in Phase I. In Phase II, the added reagents separated from the solvent phase to make fine oily droplets prior to the reaction with amines. Therefore, almost all of the reaction must proceed inside the hydrophobic domain constituted by acid anhydride. Moreover, the ln r value must be determined by the relative concentration of substrates transferred from an aqueous phase by partition. The results shown in Figs. 4 and 5 suggest that the formation of a hydrophobic domain in the aqueous phase is indispensable for the effective distinction of

TABLE 1. THE ln r VALUES OBTAINED IN THE COMPETITIVE ACYLATION OF UNBRANCHED ALKYLAMINE WITH ISOVALERIC ANHYDRIDE IN WATER–DIOXANE MIXTURES ($\chi_{H_2O} = 0.88$)[a]

	R^2–NH_2			
R^1–NH_2	CH_3CH_2–NH_2	$CH_3(CH_2)_2$–NH_2	$CH_3(CH_2)_3$–NH_2	$CH_3(CH_2)_4$–NH_2
$CH_3(CH_2)_2$–NH_2	0.330	—	—	—
$CH_3(CH_2)_3$–NH_2	0.536	0.167 (0.13)	—	—
$CH_3(CH_2)_4$–NH_2	0.863	0.418 (−0.10)	0.205	—
$CH_3(CH_2)_5$–NH_2	1.023	0.621 (0.04)	0.357	0.164

a) The concentration of a regent was 50 mM. The results of the competitions where the *N*-acetyl-L-isoleuine *N*-hydroxysuccinimide ester was used as a regent are given in parentheses.

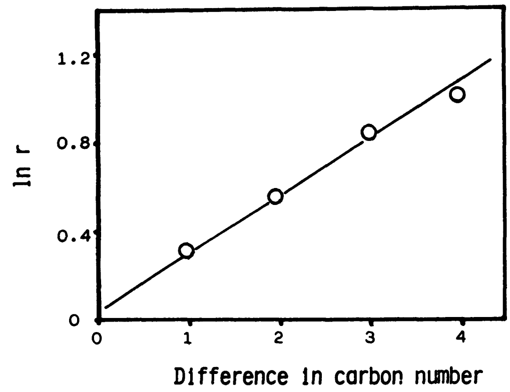


Fig. 10. The relationship between ln *r* and the difference in carbon number of alkyl chain of substrates in the competition of unbranched alkylamine and ethylamine.

alkyl-chain length.

In general, the hydrophobicity of a compound is evaluated from its partition constant between water and a certain organic solvent (*i.e.*, the π value).[12] Figure 10 shows the relationship between the ln *r* value and the difference in carbon number between alkyl groups in the competition with ethylamine. The ln *r* value has been linearly correlated with the difference in the carbon numbers of the hydrocarbon residues.[5a] Thus, the ln *r* values in Phase II can be well explained by the hydrophobicity of hydrocarbon groups.

In the competition of phenethylamine with hexylamine, the slope of the plot in Phase II showed a negative gradient, while in the competition with other, shorter alkylamines, the slopes of plots were positive (Fig. 6(b)). This result can be explained by the published finding that the phenylethyl group is more hydrophobic than the butyl group and less hydrophobic than the hexyl group.[5a]

In the results shown in Fig. 6(b), it is also worthy of note that the slopes of the plots in Phase I are all positive, regardless of their variation from positive to negative in Phase II. These results indicate that the alkyl-phenyl interaction resulting in the differentiation in Phase I has a different character from a conventionally stated hydrophobicity contributing to the differentiation in Phase II.[3,5]

In this study, we have shown that there are two different modes of the differentiation of hydrocarbon groups in the reaction in aqueous media. One is the differentiations is based on the partition of substrates between an aqueous solvent and the hydrophobic aggregates of reagents. The other is the differentiation based on characteristic molecular interactions between homogeneously dissolved substrates and reagents, such as the phenyl-phenyl and alkyl-phenyl interactions. In contrast, the molecular interaction between alkyl groups (an alkyl-alkyl interaction) is not so large as to contribute to this mode of differentiation.

The formation of a hydrophobic domain by the assembly of certain amounts of reagents is necessary for the differentiation of substrates carrying relatively short alkyl chains, since the alkyl-alkyl interaction is weak. This is an important part of understanding the role of the hydrophobic domain in macromolecules such as protein in the differentiation of the molecules carrying small-size alkyl groups. It has been reported that the rate of acylation was remarkably enhanced in an aqueous solvent when both a reagent and a substrate carried a long alkyl group.[9] This phenomenon was interpreted in terms of the "proximity effect" caused by hydrophobic bonding between hydrocarbon residues in aqueous media. However, further, critical reexaminations should be made to prove this proximity effect, as has been pointed out by Guthrie.[10] At least, the results of the present study indicate that the proximity effect is not generally applicable. Especially its contribution to the differentiation of short and medium-size alkyl groups must be negligible.

The differentiation of a phenyl group from an alkyl one could take place to a certain extent by means of a soluble reagent under homogeneous conditions. The present results suggest that aromatic hydrocarbon residues in protein not only provide a hydrophobic domain, but also have some additional functions in the differentiation of hydrocarbon residues. Several facts in the biochemical field support our results. For example, the importance of Trp or Tyr residues in the interfacial recognition site of phospholipase A_2 is well documented.[13] It has also been shown that many aromatic amino acids are neatly located along the antigen binding site of the Bence Jones protein Mcg.[14]

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