Transacylation Rates of (Aminoacyl)adenosine Moiety at the 3′-Terminus of Aminoacyl Transfer Ribonucleic Acid†

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ABSTRACT: The rates of migration of the aminoacyl group (transacylation) between 2′-*O*-(aminoacyl)-tRNA and 3′-*O*-(aminoacyl)-tRNA were studied by the nuclear magnetic resonance (NMR) analyses of 3′-terminal fragment models, with regard to the significance of transacylation in the process of protein biosynthesis. 2′(3′)-*O*-L-Alanyladenosine, -valyladenosine, -isoleucyladenosine, -phenylalanyladenosine, and -methionyladenosine, and 2′(3′)-*O*-L-phenylalanyladenosine 5′-phosphate and methionyladenosine 5′-phosphate were chemically synthesized, and the rates of transacylation in deuterated buffer were directly measured by the NMR saturation transfer method. The dependences of transacylation rates on p^2H and temperature were analyzed. The results indicate that the transacylation rates are significantly affected by the ionization states of the α-amino group of the amino acid moiety but not by the presence of the 5′-phosphate group of the adenylate moiety. The second-order rate constants for the base-catalyzed transacylation reactions were also determined for the ionized form (with α-$N^2H_3^+$ group) of (aminoacyl)-adenosines. The transacylation rates of (aminoacyl)adenosines in ^1H_2O solution at p^1H 7.3 and 37 °C (intracellular environment) were evaluated as 3–11 s^{-1} for the 2′ → 3′ transacylation and 1–4 s^{-1} for the 3′ → 2′ transacylation, indicating that the transacylation rate of *free* aminoacyl-tRNA is slower than the overall rate of polypeptide chain elongation per ribosome. This suggests the presence of some enzymatic factor for enhancing the transacylation rates of aminoacyl-tRNAs in the polypeptide chain elongation process in vivo.

In the process of protein biosynthesis, an aminoacyl-tRNA (aa-tRNA)[1] exists in two isomeric forms, namely, 2′-O-aminoacylated isomer and 3′-O-aminoacylated isomer, where the amino acid forms an ester bond with the 2′-hydroxyl group and 3′-hydroxyl group, respectively, of the 3′-terminal adenosine residue of tRNA. However, the rates of 2′ ⇌ 3′ transacylation of aminoacyl-tRNAs have not been directly determined yet. Only for formyladenosine has the transacylation rate in aqueous solution (at pH 7 and 37 °C) been estimated as about 4×10^3 s^{-1}, with several assumptions, from the experimental value for the transacylation rate of acetyluridine (Griffin et al., 1966). If the transacylation rate is indeed as fast as estimated previously, it should be impractical to determine experimentally which isomer of aa-tRNA is required in individual steps of the protein biosynthesis process. Accordingly, the aminoacylation experiments have been carried out by the use of tRNA analogues, namely, tRNAs terminating in 2′-deoxyadenosine, 3′-deoxyadenosine, 2′-deoxy-2′-aminoadenosine, or 3′-deoxy-3′-aminoadenosine (Hecht, 1979; Sprinzl & Wagner, 1979). Thus, with cognate aminoacyl-tRNA synthetases, some amino acids are charged only to the 3′-hydroxyl group, some only to the 2′-hydroxyl group, and the others to the 2′- or 3′-hydroxyl groups of such tRNA analogues (Hecht, 1979; Sprinzl & Wagner, 1979). However, for the pair of 2′-O-aminoacylated isomer (2′-isomer) and 3′-O-aminoacylated isomer (3′-isomer) of aminoacylated tRNA analogues, the 2′-isomer has been found to be predominant in the elongation factor Tu dependent binding to the A site of ribosome (Wagner et al., 1982). On the other hand, the 3′-isomer rather than the 2′-isomer of such tRNA analogues has been found to be required as the substrate of peptidyltransferase at the A site of ribosome (Sprinzl & Wagner, 1979). This means that, in each cycle of the polypeptide chain elongation (incorporation of one amino acid to the C terminus of a growing polypeptide chain), the transacylation between the two isomers of aa-tRNA should be involved and, accordingly, the rate of such a transacylation should be faster than the rate of polypeptide chain elongation per ribosome, which is 15–20 amino acid residues/s for *Escherichia coli* at 37 °C (Lacroute & Stent, 1968; Forchhammer & Lindahl, 1971).

In our previous study (Taiji et al., 1981), we have directly measured the transacylation rates of 2′(3′)-*O*-L-phenylalanyladenosine (Phe-Ado) by the NMR saturation transfer method. Surprisingly, the transacylation rates of Phe-Ado are found to be appreciably slower than the rate of polypeptide chain elongation per ribosome. In the present study, we have further analyzed the effects of the 5′-phosphate group, hydrophobic amino acid side chains, and the α-amino group on the transacylation rates. Biological implication of the slow transacylation rates of *free* aa-tRNA will be discussed in relation with the presence of some enzymatic factor that enhances the transacylation of aa-tRNA in the protein biosynthesis.

Experimental Procedures

Materials. Adenosine, adenosine 5′-phosphate (Ado-5′-P), *N-tert*-butyloxycarbonyl-L-amino acids, and [$^2H_{11}$]Tris were purchased from Kojin Co. Ltd., Yamasa Shoyu Co. Ltd., Protein Research Foundation, and Merck Sharp & Dohme Canada Ltd., respectively, and were used without further purification. For each of alanine, valine, isoleucine, phenylalanine, and methionine, aa-Ado was synthesized by the use of the condensation reaction of *N-tert*-butyloxycarbonyl-L-amino acid, rather than *N*-benzyloxycarbonyl-L-amino acid (Jonák et al., 1980), with 5′-*O*-(dimethoxytrityl)adenosine in the presence of *N*,*N*′-dicyclohexylcarbodiimide. For each of phenylalanine and methionine, aa-Ado-5′-P was synthesized

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[1] Abbreviations: aa-tRNA, aminoacyl-tRNA; aa-Ado, 2′(3′)-*O*-L-(aminoacyl)adenosine; aa-Ado-5′-P, 2′(3′)-*O*-L-(aminoacyl)adenosine 5′-phosphate; 2′-isomer, 2′-O-aminoacylated isomer; 3′-isomer, 3′-O-aminoacylated isomer; Ado-5′-P, adenosine 5′-phosphate; NMR, nuclear magnetic resonance.

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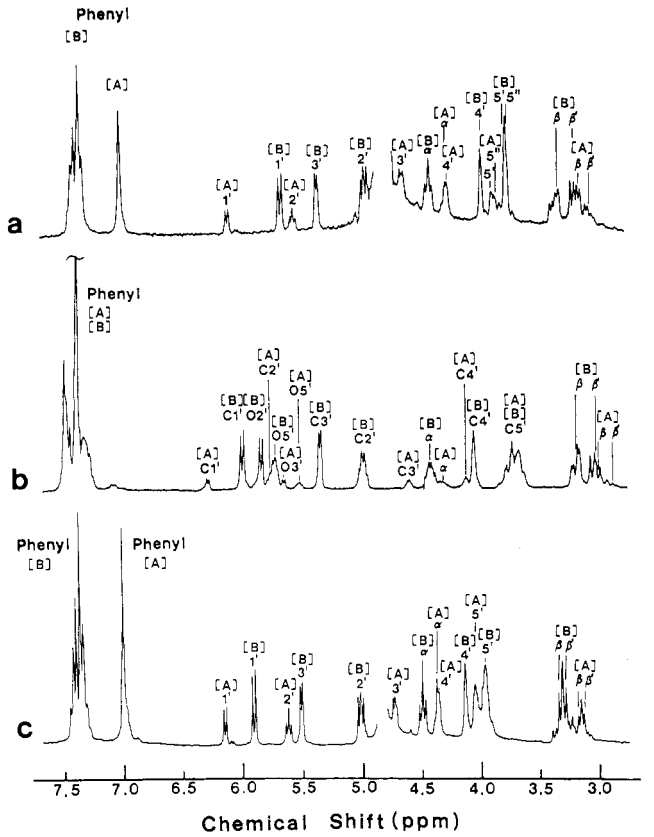

FIGURE 1: 270-MHz proton NMR spectra of (a) Phe-Ado (20 mM) in 0.5 M deuterated phosphate buffer at p^{2}H 7.3 and 25 °C, (b) (*N-tert*-butyloxycarbonyl-L-phenylalanyl)adenosine (20 mM) in $(C^2H_3)_2SO$ at 25 °C, and (c) Phe-Ado-5′-P (20 mM) in 0.5 M deuterated phosphate buffer at p^{2}H 7.1 and 25 °C. The assignments of proton resonances of the ribose moiety and amino acid moiety are also shown for the 2′-isomer [A] and 3′-isomer [B].

by the reaction of *N-tert*-butyloxycarbonyl-L-amino acid with carbonyldiimidazole and subsequently with Ado-5′-P (Azhayev et al., 1977). The purity of the synthesized product was checked by silica gel thin-layer chromatography in the system 2-butanol–formic acid–water (6:1:1) for aa-Ado and the system 1-butanol–acetic acid–water (5:2:3) for aa-Ado-5′-P and finally by proton NMR spectroscopy.

Methods. The sample solutions for NMR measurements were prepared in deuterated phosphate buffer (0.5 M) for the p^{2}H range 6.6–7.8 or in [$^2H_{11}$]Tris-^{2}HCl buffer (0.5 M) for the p^{2}H range 8.2–8.6 (the isotope correction of 0.4 was added to the pH meter reading). The pH was measured by the use of a Radiometer PHM26 pH meter with a long thin combination electrode. The 270-MHz proton NMR spectra were recorded on a Bruker WH270 spectrometer. Chemical shifts were measured relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate. Spin–lattice relaxation times (T_1) were obtained by the standard 180°–τ–90° pulse sequence. Then, the saturation transfer method (Forsén & Hoffman, 1963) was used for determining the transacylation rate from the 2′-isomer to the 3′-isomer with the following equation:

$$[I_0(2') - I(2')]/I_0(2') = k(2'\rightarrow 3')/[k(2'\rightarrow 3') + 1/T_1(2')] \quad (1)$$

$I_0(2')$ and $I(2')$ are the resonance intensities of a proton of the 2′-isomer on the off-resonance irradiation and on-resonance irradiation of the corresponding proton of the 3′-isomer, respectively. Conversely, upon irradiation of the proton of the 2′-isomer, the transacylation rate $k(3'\rightarrow 2')$ may be determined. The equilibration rate is given as $k(2'\rightleftharpoons 3') = k(2'\rightarrow 3') + k(3'\rightarrow 2')$ (Taiji et al., 1981).

Table I: Proton Chemical Shifts (ppm) at p^{2}H 7.3 and 25 °C

		ribose					
	isomer	H1′	H2′	H3′	H4′	H5′	H5″
Ala-Ado	2′	6.30	5.71	4.7	4.3	3.9[c]	3.9[c]
	3′	6.10	5.06	5.55	4.48	3.91	3.88
Val-Ado	2′	6.30	5.76	4.7	4.32	3.9[c]	3.9[c]
	3′	6.08	5.08	5.57	4.47	3.92	3.88
Ile-Ado	2′	6.29	5.76	4.72	4.32	3.95[c]	3.95[c]
	3′	6.07	5.08	5.57	4.46	3.87[c]	3.87[c]
Phe-Ado	2′	6.12	5.57	4.65	4.27	3.91	3.85
	3′	5.67	4.97	5.37	3.98	3.79	3.76
Phe-Ado-5′-P[a]	2′	6.16	5.63	4.74	4.36	4.06[c]	4.06[c]
	3′	5.92	5.03	5.53	4.14	3.97[c]	3.97[c]
Met-Ado	2′	6.32	5.77	4.72	4.3	3.9[c]	3.9[c]
	3′	6.09	5.07	5.56	4.50	3.92	3.89
Met-Ado-5′-P[b]	2′	6.35	5.75	4.77	4.43	4.09[c]	4.09[c]
	3′	6.17	5.12	5.64	4.58	4.03[c]	4.03[c]

[a] At p^{2}H 7.1. [b] At p^{2}H 7.4. [c] Indistinguishable between H5′ and H5″.

Results

Assignments of Proton Resonances. The 270-MHz proton NMR spectrum of Phe-Ado in deuterated phosphate buffer at p^{2}H 6.9 has been shown previously (Taiji et al., 1981). The NMR spectrum of Phe-Ado in deuterated phosphate buffer at a higher p^{2}H of 7.3 is shown in Figure 1a. Still, the proton resonances of the ribose moiety and amino acid moiety are observed separately for the 2′-isomer [A] and the 3′-isomer [B]. The assignments of these proton resonances to the two isomers have been explained previously (Taiji et al., 1981). As the p^{2}H is raised from 6.9 to 7.3, the α-proton resonances of the two isomers are both shifted upfield, because of the dissociation of $^2H^+$ ion from the α-$N^2H_3^+$ group of the amino acid moiety. Figure 1b shows the proton NMR spectrum of an intermediate compound in the chemical synthesis of Phe-Ado, 2′(3′)-*O*-(*N-tert*-butyloxycarbonyl-L-phenylalanyl)-adenosine in $(C^2H_3)_2SO$ solution at 25 °C. In this solution, OH proton resonances are observed at 5.87, 5.75, 5.68, and 5.55 ppm, in addition to the C–H proton resonances that are also observed in deuterated phosphate buffer solution. By the double-resonance experiments, the OH proton resonance at 5.87 ppm (Figure 1b) is found to be coupled with the C2′ proton resonance at 5.01 ppm and, thus, is assigned to the O2′ proton of the 3′-isomer [B]. Similarly, the proton resonances at 5.68 (O3′) and 4.63 ppm (C3′) are assigned to the 2′-isomer [A]. The OH proton resonances at 5.55 and 5.75 ppm are coupled with the C5′ proton resonances around 3.7 ppm and, accordingly, are assigned to the O5′ protons of the 2′-isomer and 3′-isomer, respectively. Such analyses of the proton resonances for the 2′-isomer and 3′-isomer of 2′(3′)-*O*-(*N-tert*-butyloxycarbonyl-L-phenylalanyl)adenosine further confirm the assignments of proton resonances of Phe-Ado as shown in Figure 1a.

The proton resonances of Ala-Ado, Val-Ado, Ile-Ado, and Met-Ado have also been assigned as shown in Table I (ribose protons) and Table II (base protons and amino acid protons). The proton NMR spectrum of Phe-Ado-5′-P in deuterated phosphate buffer at p^{2}H 7.1 is shown in Figure 1c. The H5′ and H5″ proton resonances are naturally shifted downfield by the phosphorylation of the 5′-CH_2OH group of Phe-Ado. Furthermore, for the 3′-isomer, rather than for the 2′-isomer, of Phe-Ado, the ribose H1′, H3′, and H4′ chemical shifts are also appreciably affected by the 5′-phosphorylation. The proton chemical shifts of Phe-Ado-5′-P and Met-Ado-5′-P are shown in Tables I and II.

Abundance Ratio of 2′-Isomer and 3′-Isomer. For aa-Ado and aa-Ado-5′-P, the abundance ratio of the 2′-isomer and

Table II: Proton Chemical Shifts (ppm) at p^{2}H 7.3 and 25 °C

	isomer	adenine H2	adenine H8	amino acid α	β	γ	δ	ε
Ala-Ado	2′	8.19	8.32	4.25	1.59			
	3′	8.19	8.32	4.34	1.66			
Val-Ado	2′	8.19	8.34	4.00	2.31	0.97		
	3′	8.19	8.32	4.13	2.43	1.11		
Ile-Ado	2′	8.18	8.35	4.08	2.00	0.91, 1.5	0.85	
	3′	8.18	8.32	4.23	2.15	1.07, 1.5	1.00	
Phe-Ado	2′	8.17	8.23	4.29	3.08, 3.18		7.02[c]	
	3′	8.17	8.23	4.42	3.19, 3.36		7.39[c]	
Phe-Ado-5′-P[a]	2′	8.20	8.54	4.38	3.14, 3.20		7.01[c]	
	3′	8.20	8.55	4.50	3.29, 3.36		7.39[c]	
Met-Ado	2′	8.19	8.36	4.18	2.3	2.57		1.94
	3′	8.19	8.32	4.32	2.3	2.74		2.16
Met-Ado-5′-P[b]	2′	8.22	8.56	4.12	2.3	2.53		1.94
	3′	8.22	8.56	4.29	2.3	2.75		2.16

[a] At p^{2}H 7.1. [b] At p^{2}H 7.4. [c] Highest peak of phenyl protons.

Table III: Equilibrium Constant, Enthalpy (kcal mol^{-1}), and Entropy (cal mol^{-1} K^{-1}) Differences between 2′-Isomer and 3′-Isomer

	equilibrium const[a]	enthalpy difference	entropy difference
Ala-Ado	0.36[b]	−0.4 ± 0.3	−3.5 ± 2.7
Val-Ado	0.37[b]	−0.2 ± 1.0	−2.6 ± 3.2
Ile-Ado	0.39[b]	0.0 ± 0.0	−1.9 ± 0.0
Phe-Ado	0.40[b]	−0.4 ± 0.3	−3.2 ± 0.8
Phe-Ado-5′-P	0.47[c]		
Met-Ado	0.42[b]		
Met-Ado-5′-P	0.47[d]		

[a] [2′-Isomer]/[3′-isomer]. [b] At p^{2}H 7.3 and 25 °C. [c] At p^{2}H 7.1 and 25 °C. [d] At p^{2}H 7.4 and 25 °C.

3′-isomer has been determined from the intensity ratio of the corresponding proton resonances of the two isomers. For Ala-Ado, Val-Ado, Ile-Ado, Phe-Ado, and Met-Ado, the abundance ratio (2′-isomer/3′-isomer) is found to be in a narrow range, 0.36–0.42 at p^{2}H 7.3 and 25 °C (Table III). The abundance ratio is slightly higher in Phe-Ado-5′-P and Met-Ado-5′-P (Table III) than in the cases of aa-Ado.

The abundance ratio 2′-isomer/3′-isomer changes little with temperature (20–35 °C), indicating that the enthalpy difference is smaller than RT (R being the gas constant). The enthalpy and entropy differences between the 2′-isomer and 3′-isomer have been obtained as shown in Table III, from the least-squares analyses of the temperature dependence of the abundance ratio 2′-isomer/3′-isomer.

Determination of Transacylation Rates by Saturation Transfer Method. We have previously succeeded (Taiji et al., 1981) in the direct determination of the transacylation rate of Phe-Ado in ^2H_2O solution at p^{2}H 6.9, by the application of the saturation transfer method to the H1′ proton resonances of the 2′- and 3′-isomers. In this Phe-Ado molecule, the chemical shift difference of the H1′ protons of the 2′- and 3′-isomers is as large as 0.45 ppm, and the peak intensities of H1′ proton resonances (doublet) are relatively high as compared with those of H2′ proton resonances (quartet or apparently triplet, Figure 1a), so that the transacylation rate may be precisely determined. In the present study, however, the transacylation rate of Phe-Ado in ^2H_2O solution at p^{2}H 7.3 has been determined by the use of the H1′ proton resonances

Table IV: Transacylation Data on Phe-Ado in ^2H_2O Solution at p^{2}H 7.3 and 25 °C

		H1′	H2′
T_1 (s)	2′-isomer	1.04	0.71
	3′-isomer	1.06	0.71
saturation transfer (%)	2′-isomer	43	32
	3′-isomer	23	16
transacylation rate (s^{-1})	k(2′→3′)	0.73	0.67
	k(3′→2′)	0.28	0.28
equilibration rate (s^{-1})	k(2′⇌3′)	1.01	0.95

Table V: Transacylation Rates (s^{-1}) in ^2H_2O Solution

	p^{2}H 7.3, 25 °C		p^{2}H 7.3, 37 °C	
	k(2′→3′)	k(3′→2′)	k(2′→3′)	k(3′→2′)
Ala-Ado	1.25	0.46	3.54	1.33
Val-Ado	0.64	0.22	2.04	0.76
Ile-Ado	0.34	0.14	1.06	0.41
Phe-Ado	0.73	0.28	2.53	1.01
Phe-Ado-5′-P	0.42[a]	0.19[a]		
Met-Ado	1.20	0.42		
Met-Ado-5′-P	1.31[b]	0.62[b]		

[a] At p^{2}H 7.1, 25 °C. [b] At p^{2}H 7.4, 25 °C.

and also of the H2′ proton resonances. For the H1′ proton of the 3′-isomer, the longitudinal relaxation time (T_1) is measured as 1.06 s, and on irradiation of the H1′ proton of the 2′-isomer, the intensity decrease (saturation transfer) of the H1′ proton resonance of the 3′-isomer is found to be 23%. Then, by the use of eq 1, the transacylation rate k(3′→2′) is obtained as 0.28 s^{-1}. Similarly, for the H2′ proton of the 3′-isomer, T_1 is measured as 0.71 s, and the saturation transfer is found to be 16%, so that the rate k(3′→2′) is obtained as 0.28 s^{-1}, in good agreement with the value as determined by the use of the H1′ proton resonances (Table IV). The saturation transfer experiments have also been carried out for the less abundant 2′-isomer of Phe-Ado. The transacylation rate k(2′→3′) = 0.67 s^{-1} as obtained from the weak H2′ proton resonance (apparently triplet, Figure 1a) still agrees well with the value of 0.73 s^{-1} as determined precisely by the use of the H1′ proton resonance (Table IV).

For aa-Ado (other than Phe-Ado) and aa-Ado-5′-P, the H1′ chemical shift differences between the 2′- and 3′-isomers are as small as 0.18–0.24 ppm (Table I), and at the resonance frequency of 270 MHz, the irradiation of the H1′ proton of one isomer directly affects the resonance intensity of the H1′ proton of the other isomer. However, for the H2′ proton resonances, the chemical shift differences between the two isomers are as large as 0.60–0.70 ppm (although the peak intensities are relatively low because of the multiplet structure). Accordingly, in the present study, H2′ proton resonances have been used in the saturation transfer experiments on aa-Ado and aa-Ado-5′-P, and rate constants (k) in ^2H_2O solution at 25 °C are listed in Table V.

Temperature Dependence of Transacylation Rates of aa-Ado. The transacylation rates of Phe-Ado in ^2H_2O solution have been determined, by the saturation transfer method, at 20, 25, 30, and 37 °C, where the p^{2}H is kept constant at 7.3. As the temperature is raised, the transacylation rates, k(2′→3′) and k(3′→2′), are appreciably enhanced, and their Arrhenius plots are shown in Figure 2. The experimental data lie close to the straight lines (Figure 2) as obtained by the least-squares method. Thus, the apparent enthalpy of activation is obtained as $\Delta H^{\ddagger}$(2′→3′) = 18.7 ± 0.7 kcal mol^{-1} for the 2′ → 3′ transacylation and $\Delta H^{\ddagger}$(3′→2′) = 19.1 ± 0.8 kcal mol^{-1} for the 3′ → 2′ transacylation. It may be noted here that the sum of the enthalpy difference ΔH(2′–3′) (Table III) and the en-

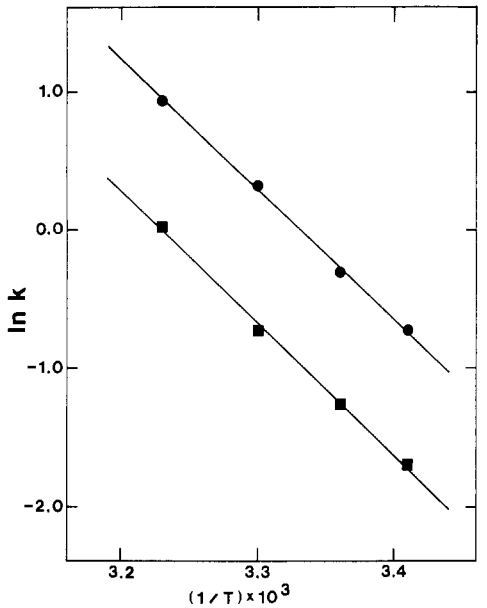

FIGURE 2: Arrhenius plots of transacylation rates, $k(2'\rightarrow3')$ (●) and $k(3'\rightarrow2')$ (■), of Phe-Ado in ^2H_2O solution at p^{2}H 7.3.

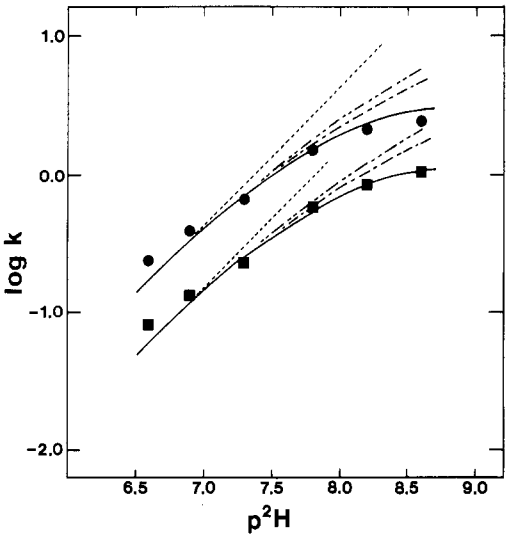

FIGURE 3: p^{2}H dependences of transacylation rates, $k(2'\rightarrow3')$ (●) and $k(3'\rightarrow2')$ (■), of Val-Ado in ^2H_2O solution at 25 °C. The theoretical curves for log k_{obsd} vs. p^{2}H are also shown for the cases where the ratios k^+/k^0 are equal to 1 (---), 5 (–·––), 10 (–··–), and 100 (—).

thalpy of activation $\Delta H^{\ddagger}(2'\rightarrow3')$ is to be equal to the enthalpy of activation $\Delta H^{\ddagger}(3'\rightarrow2')$. In fact, for Phe-Ado, the value of $\Delta H^{\ddagger}(2'\rightarrow3')$ + $\Delta H(2'-3')$ is obtained as 18.3 ± 0.8, in agreement with the value of $\Delta H^{\ddagger}(3'\rightarrow2')$. From the Arrhenius plots of transacylation rates, $\Delta H^{\ddagger}(2'\rightarrow3')$ values have been obtained as 16 ± 4 kcal mol^{-1} (Ala-Ado), 18 ± 1 kcal mol^{-1} (Val-Ado), and 17 ± 1 kcal mol^{-1} (Ile-Ado) and $\Delta H^{\ddagger}(3'\rightarrow2')$ values as 16 ± 3 kcal mol^{-1} (Ala-Ado), 19 ± 1 kcal mol^{-1} (Val-Ado) and 16 ± 1 kcal mol^{-1} (Ile-Ado).

p^{2}H Dependence of Transacylation Rates of aa-Ado. The transacylation rates of Val-Ado in ^2H_2O solution at 25 °C have been measured over the p^{2}H region 6.6–8.6. The transacylation rates obtained in [$^2H_{11}$]Tris-^{2}HCl buffer at p^{2}H 7.3 are confirmed to be equal to those obtained in deuterated phosphate buffer at the same p^{2}H. As shown in Figure 3, the transacylation rates, $k(2'\rightarrow3')$ and $k(3'\rightarrow2')$, are increased as the p^{2}H is raised, indicating that these transacylation reactions are base catalyzed. However, the values of log $k(2'\rightarrow3')$ and log $k(3'\rightarrow2')$ do not change linearly with p^{2}H in the region 6.6–8.6. On the other hand, from the p^{2}H dependence of the C_α proton chemical shifts of the 2'- and 3'-isomers of Val-Ado in ^2H_2O solution at 25 °C, the pK_a values for the ionization of the α-amino group of the two isomers are both found to be 7.9. These observations indicate that the rate of transacylation (k^+) of the ionized form (with α-N$^2H_3^+$ group) is different from the rate of transacylation (k^0) of the unionized form (with α-N$^2H_2$ group).

Discussion

Equilibrium between 2'-Isomer and 3'-Isomer of aa-Ado. From the temperature dependence of equilibrium constants, [2'-isomer]/[3'-isomer], the enthalpy difference (ΔH) between the two isomers is found to be smaller than RT for each of Ala-Ado, Val-Ado, Ile-Ado, and Phe-Ado (Table III). The 2'-isomer is slightly more stable than the 3'-isomer, but the 3'-isomer is more abundant than the 2'-isomer.

For the 2'-isomer, rather than the 3'-isomer, of Ile-Ado, some hydrophobic interaction may well be expected between the bulky side chain and the adenine ring. On the other hand, for Ala-Ado, such a hydrophobic interaction is not expected to be significant even for the 2'-isomer, because of the small size of the side-chain methyl group. However, the entropy differences for Ala-Ado and Ile-Ado (Table III) are not much different from each other, suggesting that the side-chain groups of the amino acid moiety do not interact strongly with the adenine ring of the nucleoside moiety. Therefore, the larger entropy of the 3'-isomer as compared with the 2'-isomer is probably due to the difference in the flexibility of the ribose ester moiety of the two isomers. In fact, the equilibrium constant [2'-isomer]/[3'-isomer] of acetyluridine (0.6) (Griffin et al., 1966) is not much different from those of aa-Ados as determined in the present study.

Effect of 5'-Phosphate Group on Conformation of aa-Ado-5'-P. The presence of the 5'-phosphate group in Met-Ado-5'-P and Phe-Ado-5'-P slightly increases the equilibrium constants [2'-isomer]/[3'-isomer] (Table III), as compared with those for Met-Ado and Phe-Ado, respectively. For each of the 2'- and 3'-isomers, the chemical shifts of the ribose-ring protons (H1', H2', H3', and H4') and adenine H2 proton of Met-Ado-5'-P are nearly the same as those of Met-Ado. Only for the adenine H8 proton are significant downfield shifts observed of the 2'- and 3'-isomers of Met-Ado-5'-P, as compared with those of Met-Ado (Tables I and II). Here it may be recalled that Ado takes the syn form as well as the anti form while Ado-5'-P primarily takes the anti form (Son & Chachaty, 1973). Thus, the presence of the 5'-phosphate group converts the syn form of the adenosine moiety to the anti form and then possibly affects the chemical shift of the adenine H8 proton in the proximity. A similar downfield shift of the adenine H8 proton resonance is also observed of Phe-Ado-5'-P, as compared with Phe-Ado (Table II). However, the presence of the 5'-phosphate group of Met-Ado-5'-P and Phe-Ado-5'-P does not affect the proton chemical shifts of the amino acid moiety, as compared with the corresponding resonances of Met-Ado and Phe-Ado, respectively (Table II). These observations indicate that the amino acid moiety of aa-Ado-5'-P does not interact significantly with the phosphate group at the 5'-position of the adenosine moiety.

Effect of 5'-Phosphate Group on Transacylation Rates of aa-Ado-5'-P. The transacylation rate of Met-Ado-5'-P is slightly faster than that of Met-Ado whereas the transacylation rate of Phe-Ado-5'-P is slightly slower than that of Phe-Ado (Table V). Thus, the presence of the 5'-phosphate group does not affect the transacylation rates of aa-Ado-5'-P in a sys-

tematic manner, although it does stabilize the anti form of the adenosine moiety. This may be understood reasonably, since the adenine ring lies on the same side of the ribose ring as the 5′-phosphate group whereas the transacylation site (around the O2′ and O3′ atoms) lies on the opposite side of the ribose ring to the 5′-phosphate group. Accordingly, the stacking of the adenine ring, if any, of the CCA terminus in aa-tRNA (Cheng et al., 1980) will not significantly affect the rate of transacylation between the 2′- and 3′-isomers.

Effect of Amino Acid Side Groups on Transacylation Rates of aa-Ado. In contrast to the 5′-phosphate group or the adenine ring, the amino acid side group of aa-Ado lies on the same side of the ribose ring as the transacylation site around the O2′ and O3′ atoms. However, transacylation rates are not systematically affected by the bulkiness of hydrophobic side groups (Table V). The transacylation rates are in the order Ala-Ado > Met-Ado > Phe-Ado > Val-Ado > Ile-Ado; within the narrow ranges, $k(2'\rightarrow3') = 1.3$–0.3 s^{-1} and $k(3'\rightarrow2') = 0.5$–0.1 s^{-1} at around p^2H 7.3 and 25 °C. The apparent $\Delta H^{\ddagger}$ values for Ala-Ado, Val-Ado, Ile-Ado, and Phe-Ado are in the narrow range 16–19 kcal mol^{-1}. The amino acid side groups thus do not appear to interact significantly with the transacylation site of aa-Ado.

Effect of Ionization of α-Amino Group on Transacylation Rates. The p^2H dependence of the transacylation rates of Val-Ado in ^2H_2O solution indicates that the transacylation rates (k^+) of the ionized form (with α-$N^2H_3^+$ group) are different from the transacylation rates (k^0) of the un-ionized form (with α-N^2H_2 group). Then, the observed transacylation rate k_{obsd} is to be given as follows:

$$k_{obsd} = [O^2H^-](k^+[^2H^+] + k^0K_a)/([^2H^+] + K_a) \quad (2)$$

For the pK_a value of 7.9 (Val-Ado at 25 °C), the theoretical dependence of log k_{obsd} on p^2H is shown in Figure 3, for the four cases where the ratio k^+/k^0 is equal to 1, 5, 10, or ≥100. If $k^+/k^0 = 1$, the value of log k_{obsd} should be proportional to p^2H. However, if $k^+/k^0 = 5$ or 10, the value of log k_{obsd} vs. p^2H will deviate appreciably downward from linearity. For Val-Ado in ^2H_2O solution, the p^2H dependence of log $k(2'\rightarrow3')$ and log $k(3'\rightarrow2')$ is found to agree closely with the theoretical curves for the case $k^+/k^0 \geq 100$. Similarly for Ala-Ado, Ile-Ado, and Phe-Ado in ^2H_2O solution at 25 °C, the ratio of transacylation rates k^+/k^0 is also found to be ≥100. Such an enhanced rate of the ionized form is certainly expected for a base-catalyzed reaction, since the presence of the positively charged α-$N^2H_3^+$ group in the proximity to the ester bond possibly stabilizes the negatively charged intermediate form of the ester group.

Because of the high ratio of k^+/k^0, the transacylation rates are nearly proportional to p^2H up to around 7 (see the solid lines in Figure 3), which are largely due to the ionized form. The contribution of the un-ionized form to the transacylation rates will become comparable to that of the ionized form only after the p^2H is raised to $pK_a + \log 100 \simeq 9.9$, and thereafter, the transacylation rates will tend to depend more linearly with $[O^2H^-]$.

In relation with the $2' \rightleftharpoons 3'$ transacylation reactions as analyzed in the present study, the deacylation reactions have been studied previously for Val-tRNA (Schuber & Pinck, 1974), Leu-tRNA (Wolfenden, 1963), Thr-tRNA, His-tRNA, and Phe-tRNA (Gatica et al., 1966). For these aa-tRNAs, the pH dependences of deacylation rates have been observed, and the deacylation rates of the ionized form (with α-NH_3^+ group) have been found to be about 100 times as fast as those of the un-ionized form (with α-NH_2 group). Furthermore, the deacylation rate constants k^+ and k^0 (eq 2) of aa-tRNAs in ^1H_2O solution have been found to be about 0.8 times as fast as those in ^2H_2O solution, just as expected for base-catalyzed reactions (Schuber & Pinck, 1974). As compared with such deacylation reactions, the transacylation reactions of aa-Ado (and 5′-phosphates) are much faster. However, such transacylation reactions are also found, in the present study, to be enhanced by the presence of the ionized α-amino group of the amino acid moiety.

Second-Order Rate Constants in ^2H_2O Solution. The rate constants $k^+(2'\rightarrow3')$ and $k^+(3'\rightarrow2')$ of the ionized form of Val-Ado in ^2H_2O solution may be estimated by the use of eq 2 for the case $k^+/k^0 \geq 100$ (Figure 3). From the pK_a (7.9 at 25 °C) and the ion product of ^2H_2O (14.87 at 25 °C; Weast, 1979), the second-order rate constants of Val-Ado are estimated as $k^+(2'\rightarrow3') = 3.0 \times 10^7$ s^{-1} M^{-1} and $k^+(3'\rightarrow2') = 1.0 \times 10^7$ s^{-1} M^{-1} at 25 °C. For the estimation of rate constants at higher temperatures, the pK_a values of the α-amino group of Val-Ado have been obtained as 7.8 at 30 °C and 7.6 at 37 °C, by using the enthalpy difference (10 kcal mol^{-1}) of aminoacyl moieties (Sober, 1968). Then, from the transacylation rates (Table V) and the ion product (14.70 at 30 °C and 14.48 at 37 °C; Weast, 1979), the second-order rate constants are estimated as $k^+(2'\rightarrow3') = 3.6 \times 10^7$ s^{-1} M^{-1} at 30 °C and 4.6×10^7 s^{-1} M^{-1} at 37 °C and $k^+(3'\rightarrow2') = 1.4 \times 10^7$ s^{-1} M^{-1} at 30 °C and 1.7×10^7 s^{-1} M^{-1} at 37 °C. The temperature dependences of these second-order rate constants (k^+) yield the enthalpy of activation as $\Delta H^{\ddagger}(2'\rightarrow3') = 7$ kcal mol^{-1} and $\Delta H^{\ddagger}(3'\rightarrow2') = 8$ kcal mol^{-1}. These $\Delta H^{\ddagger}$ values are appreciably lower than the *apparent* $\Delta H^{\ddagger}$ values (18–19 kcal mol^{-1}) as obtained from the temperature dependences of transacylation rates at *constant* p^2H. This indicates the importance of the corrections for temperature dependences of the ion product of ^2H_2O and also the pK_a of the α-amino group. The second-order rate constants $k^+(2'\rightarrow3')$ and $k^+(3'\rightarrow2')$ have also been estimated as 7.9×10^7 s^{-1} M^{-1} and 3.0×10^7 s^{-1} M^{-1} (Ala-Ado), 2.4×10^7 s^{-1} M^{-1} and 0.9×10^7 s^{-1} M^{-1} (Ile-Ado), and 5.7×10^7 s^{-1} M^{-1} and 2.3×10^7 s^{-1} M^{-1} (Phe-Ado), respectively, in ^2H_2O solution at 37 °C.

Transacylation Rates of aa-Ado in ^1H_2O Solution. The transacylation rates of aa-Ados as measured in ^2H_2O solution may be used for estimating the transacylation rates of aa-Ados in ^1H_2O solution, for example, at p^1H 7.3 and 37 °C [the intracellular environment in *E. coli* (Ugurbil et al., 1979)]. As shown in eq 2, transacylation rates depend on the concentration of O^1H^- ion and the ionization state of the α-amino group of the amino acid moiety. The pK_a value of the amino group is known to be 0.5 unit lower in ^1H_2O solution than in ^2H_2O solution (Jencks & Carriuolo, 1960), so that the pK_a of Val-Ado in ^1H_2O solution at 37 °C is obtained as 7.1 from the pK_a (7.6 at 37 °C) in ^2H_2O solution. Since $k^+/k^0 \geq 100$ for Val-Ado, the transacylation rates at p^1H 7.3 are predominantly due to the ionized form (with α-NH_2 group) as shown in eq 3. As for a base-catalyzed reaction, the rate constant

$$k_{obsd} = [O^1H^-](k^+[^1H^+])/([^1H^+] + K_a) \quad (3)$$

in ^1H_2O solution is about 0.8 times as large as that in ^2H_2O solution (Englander et al., 1972; Schuber & Pinck, 1974). Thus, the second-order rate constants of Val-Ado in ^1H_2O solution are obtained as $k^+(2'\rightarrow3') = 3.6 \times 10^7$ s^{-1} M^{-1} and $k^+(3'\rightarrow2') = 1.4 \times 10^7$ s^{-1} M^{-1} at 37 °C, from the corresponding rate constants in ^2H_2O solution. By the use of the ion product of ^1H_2O (13.62 at 37 °C; Weast, 1979), the transacylation rates of Val-Ado in ^1H_2O solution at p^1H 7.3 and 37 °C are thus estimated as $k(2'\rightarrow3') = 6.6$ s^{-1} and $k(3'\rightarrow2') = 2.5$ s^{-1}. Similarly, the transacylation rates $k(2'\rightarrow3')$ and $k(3'\rightarrow2')$ are obtained as 11.4 s^{-1} and 4.3 s^{-1}

(Ala-Ado), 3.4 s^{-1} and 1.3 s^{-1} (Ile-Ado), and 8.2 s^{-1} and 3.3 s^{-1} (Phe-Ado), respectively, in ^1H_2O solution at p^{1}H 7.3 and 37 °C.

Transacylation Rates of aa-tRNA. X-ray crystal analyses on yeast $tRNA^{Phe}$ have shown that the 3′-CCA terminal moiety is not involved in the formation of the secondary and tertiary structures (Sussman et al., 1978). Furthermore, RNase A digestion of aa-tRNA under a mild condition readily releases the 3′-terminal aa-Ado (Tanada et al., 1981). These observations indicate that the 3′-terminal aa-Ado moiety of aa-tRNA is exposed to the aqueous medium and is not significantly involved in the interactions with other parts of aa-tRNA molecule. Accordingly, the transacylation rates of *free* aa-tRNA are expected to be in the same range as found in the present study on aa-Ado and aa-Ado-5′-P [$k(2'\rightarrow3')$ = 3–11 s^{-1} and $k(3'\rightarrow2')$ = 1–4 s^{-1} at p^{1}H 7.3 and 37 °C].

It might be anticipated that the transacylation rates of aa-tRNA are possibly affected by the ionized side-chain groups of the amino acid moiety. However, for similar base-catalyzed reactions, namely, deacylation reactions of aa-tRNAs, the ionized side chains have been found not to affect the reaction rates. Thus, the deacylation rates of Glu-tRNA (with a negatively charged γ-COO^- group) are nearly the same as those of Met-tRNA and Phe-tRNA (Schuber & Pinck, 1974). Furthermore, the deacylation rate of Lys-tRNA (with a positively charged ϵ-NH_3^+ group) is as slow as that of Phe-tRNA (M. Hara, S. Yokoyama & T. Miyazawa, unpublished results). These observations suggest that the transacylation rates of aa-tRNA in neutral aqueous solution are not significantly affected by a variety of amino acid side groups and are as slow as those of aa-Ado and aa-Ado-5′-P.

Biological Implication of Slow Transacylation Rates of aa-tRNA. The overall rate of polypeptide chain elongation per ribosome has been reported as 15–20 amino acid residues/s for *E. coli* cells at 37 °C (Lacroute & Stent, 1968; Forchhammer & Lindahl, 1971). During the multistep reaction of polypeptide chain elongation, the specificity for 2′-isomer/3′-isomer of aa-tRNA has been elucidated (Hecht, 1979; Wagner et al., 1982). The 2′-isomer of aa-tRNA is predominant in the elongation factor Tu dependent binding to the ribosomal A site whereas only the 3′-isomer of aa-tRNA is required as the substrate of peptidyltransferase of ribosome. Previously, either isomer has been considered to be readily available in every step of polypeptide chain elongation, on the basis of fast enough spontaneous 2′ $\rightleftharpoons$ 3′ equilibration of aa-tRNA (Griffin et al., 1966). However, in the present study, the transacylation rates of aa-tRNA have been determined as $k(2'\rightarrow3')$ = 3–11 s^{-1} and $k(3'\rightarrow2')$ = 1–4 s^{-1} in ^1H_2O solution at p^{1}H 7.3 and 37 °C (intracellular environment). Surprisingly, these rates are slower than the overall rate of polypeptide chain elongation per ribosome. Thus, the spontaneous transacylation of aa-tRNA is *not* as fast as required during the polypeptide chain elongation process. Accordingly, in this process, the transacylation of aa-tRNA in vivo needs to be enhanced by some enzymatic factor probably involved in the complex with elongation factor Tu and ribosome (including peptidyltransferase).

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Registry No. Phe-Ado (2′-isomer), 25164-30-1; Phe-Ado (3′-isomer), 5956-81-0; Ala-Ado (2′-isomer), 4217-73-6; Ala-Ado (3′-isomer), 4217-74-7; Val-Ado (2′-isomer), 2319-20-2; Val-Ado (3′-isomer), 2147-07-1; Ile-Ado (2′-isomer), 85234-48-6; Ile-Ado (3′-isomer), 85248-51-7; Met-Ado (2′-isomer), 4217-71-4; Met-Ado (3′-isomer), 4217-72-5; Phe-Ado-5′-P (2′-isomer), 29839-35-8; Phe-Ado-5′-P (3′-isomer), 29839-36-9; Met-Ado-5′-P (2′-isomer), 85649-48-5; Met-Ado-5′-P (3′-isomer), 85649-49-6; 2′-*O*-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl)adenosine, 85649-50-9; 3′-*O*-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl)adenosine, 85649-51-0.

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