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# Slow Transacylation of Peptidyladenosine Allows Analysis of the 2′/3′-Isomer Specificity of Peptidyltransferase†

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ABSTRACT: 2′-*O*-(*N*-acetyl-L-phenylalanyl-L-phenylalanyl)adenosine and 3′-*O*-(*N*-acetyl-L-phenylalanyl-L-phenylalanyl)adenosine (Ac-Phe-Phe-Ado) were chemically synthesized, and these two isomers were clearly separated from each other by high-performance liquid chromatography (HPLC) on an ODS column. By this HPLC method, the abundance ratio of the 2′-isomer and 3′-isomer in equilibrium in aqueous solution at pH 7.0 and 0 °C was found to be 0.30:0.70, and the equilibration rate was determined as 0.59 ± 0.04 $min^{-1}$. Thus, the rate of transacylation between the 2′-isomer and 3′-isomer of peptidyl-tRNA was found to be much slower than that for the two isomers of aminoacyl-tRNA. The HPLC method was used for isomer analysis of the product of the *Escherichia coli* ribosomal peptidyltransferase reaction. By the use of an *isomerizable* analogue, 2′(3′)-*O*-L-phenylalanyladenosine (Phe-Ado), as the acceptor of the *N*-acetyl-L-[$^3$H]phenylalanine (Ac-[$^3$H]Phe) group in the Ac-[$^3$H]Phe-tRNA$^{Phe}$·poly(U)·70S ribosome system, the reaction product was found exclusively to be the 3′-isomer of Ac-[$^3$H]Phe-Phe-Ado. Thus, the slow transacylation of peptidyladenosine allows the analysis of the 2′/3′-isomer specificity of peptidyltransferase.

In the process of protein biosynthesis, the CCA-terminal adenosine residue of a tRNA species is aminoacylated, by the cognate aminoacyl-tRNA synthetase, to form 2′-O-aminoacylated isomer (2′-isomer)[1] or 3′-O-aminoacylated isomer (3′-isomer). The 2′-isomer and 3′-isomer of an aminoacyladenosine moiety are shown in Figure 1a. The conversion between the 2′-isomer and 3′-isomer of aminoacyl-tRNA (transacylation) has long been believed to be as fast as 4 × $10^3$ $s^{-1}$, with reference to the transacylation rate as estimated for formyladenosine in aqueous solution (Griffin et al., 1966). If the transacylation rate was as fast as estimated previously, it should be impractical to determine experimentally which isomer of aminoacyl-tRNA is required in individual steps of the protein biosynthesis process. Therefore, the 2′/3′-isomer specificities for aminoacyl-tRNA and peptidyl-tRNA have been analyzed by the use of nonisomerizable tRNA analogues, including those terminating in 2′-deoxyadenosine (2′-deoxy-Ado-tRNA) and in 3′-deoxyadenosine (3′-deoxy-Ado-tRNA) (Hecht, 1979; Sprinzl & Wagner, 1979; Wagner et al., 1982; Wagner & Sprinzl, 1983). Thus, Sprinzl and co-workers have concluded that the transacylation between the 2′-isomer and 3′-isomer is inevitably involved in the polypeptide chain elongation cycle (Sprinzl & Wagner, 1979; Wagner et al., 1982).

Actually, however, by the NMR saturation transfer method, we have succeeded in the experimental determination of the transacylation rates of aminoacyladenosines (Figure 1a) as faithful models for the CCA-terminal moiety of aminoacyl-tRNA species (Taiji et al., 1981). Surprisingly, in aqueous solution at pH 7.3 and 37 °C (intracellular environment), the transacylation rates are found to be as slow as 3-11 $s^{-1}$ for the 2′ → 3′ transacylation and 1-4 $s^{-1}$ for the 3′ → 2′ transacylation (Taiji et al., 1983). These transacylation rates are

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[1] Abbreviations: Ac-Phe, *N*-acetyl-L-phenylalanine; Ac-Phe-Phe-Ado, 2′(3′)-*O*-(*N*-acetyl-L-phenylalanyl-L-phenylalanyl)adenosine; Ac-Phe-tRNA$^{Phe}$, *N*-acetyl-L-phenylalanyl-tRNA$^{Phe}$; Ado, adenosine; Bes, 2-[bis-(2-hydroxyethyl)amino]ethanesulfonic acid; 2′-deoxy-Ado-tRNA, tRNA terminating in 2′-deoxyadenosine; 3′-deoxy-Ado-tRNA, tRNA terminating in 3′-deoxyadenosine; HONB, *N*-hydroxy-5-norbornene-2,3-dicarboximide; HPLC, high-performance liquid chromatography; 2′-isomer, 2′-O-aminoacylated isomer; 3′-isomer, 3′-O-aminoacylated isomer; Phe-Ado, 2′(3′)-*O*-L-phenylalanyladenosine; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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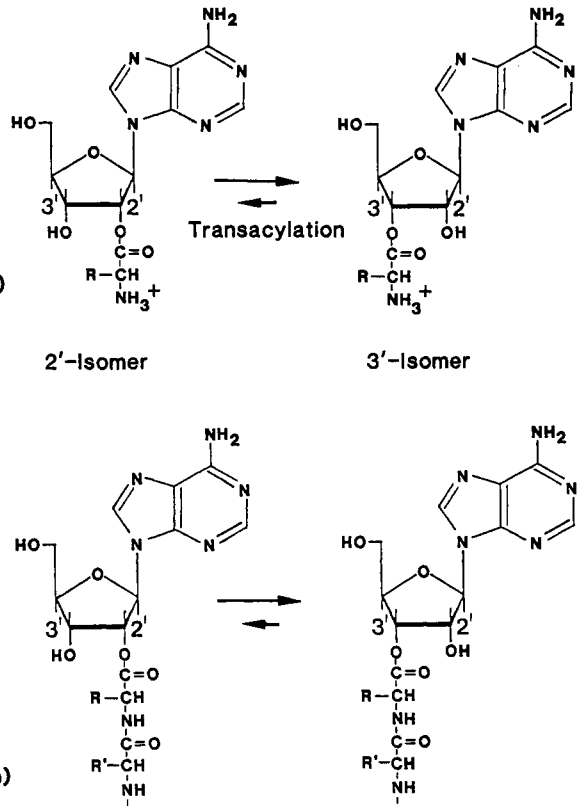


FIGURE 1: 2'-Isomer and 3'-isomer of aminoacyladenosine (a) and peptidyladenosine (b).

even slower than the overall rate (15–20 amino acid residues $s^{-1}$) of polypeptide chain elongation per ribosome in *Escherichia coli* at 37 °C (Lacroute & Stent, 1968; Forchhammer & Lindahl, 1971). These indicate the importance of reexamining the 2'/3'-isomer specificities, in the elongation cycle, as deduced from studies on nonisomerizable tRNA analogues. It may be recalled here that nonisomerizable peptidyl-tRNA analogues cannot be translocated from the A site to the P site of a ribosome, and thus the elongation cycle is not completed (Wagner & Sprinzl, 1983). Therefore, it is important to elucidate the 2'/3'-isomer specificities for native *isomerizable* species of aminoacyl-tRNA and peptidyl-tRNA.

Such analyses of 2'/3'-isomer specificities are now expected to be practical, since we have found that the transacylation rate of aminoacyladenosine with an un-ionized $\alpha$-amino group is remarkably slower than that of aminoacyladenosine with a protonated $\alpha$-amino group (Taiji et al., 1983). In fact, in the present study, we have found that the transacylation rate of peptidyladenosine (Figure 1b) is significantly slower than that of aminoacyladenosine, and the abundance ratio of the 2'-isomer and 3'-isomer in aqueous solution may be determined by high-performance liquid chromatography (HPLC). Thus, the HPLC method may be used for isomer analysis of the product of the peptidyltransferase reaction in the polypeptide chain elongation cycle.

## Experimental Procedures

*Materials.* L-Phenyl[2,3-$^3$H]alanine (33 Ci/mmol) was purchased from Amersham, and methanol (HPLC grade) and [$^2H_4$]methanol were purchased from Merck. 2'(3')-*O*-L-Phenylalanyladenosine (Phe-Ado) was chemically synthesized as described previously (Taiji et al., 1981). The phenol extract of *E. coli* Q13 was kindly provided by Dr. S. Nishimura. Purification of $tRNA^{Phe}$ was performed by the combination of chromatographic procedures with columns of DEAE-Sephadex A-50 (Pharmacia Fine Chemicals) at pH 7.0 and at pH 4.0 (Nishimura, 1971) and benzoylated DEAE-cellulose (Boehringer Mannheim GmbH) (Gillam & Tener, 1971). Phenylalanyl-tRNA synthetase (EC 6.1.1.20) was partially purified from *Thermus thermophilus* HB8 (Ha, J. M., Kohda, D., Yokoyama, S., & Miyazawa, T., unpublished results). 70S ribosome was purified from *E. coli* PR13 as described previously (Gesteland, 1966; Reiner, 1969).

*Methods.* HPLC was performed on a Toyo Soda SP8700 system equipped with a Shimadzu SHIM-PACK PC18 column (ODS, 0.46 × 5 cm). Isocratic elution was performed, and the eluate was monitored with UV absorption at 254 nm. Proton NMR spectra were recorded on Bruker WH-270 or WM-400 spectrometer. The $^3$H radioactivity was counted with an Aloka liquid scintillation system 700, with Amersham ACS II as the cocktail for aqueous scintillation counting.

*Chemical Synthesis of 2'(3')-O-(N-Acetyl-L-phenylalanyl-L-phenylalanyl)adenosine (Ac-Phe-Phe-Ado).* Ac-Phe-Phe-Ado was synthesized by the condensation of *N*-acetyl-L-phenylalanine (Ac-Phe) and Phe-Ado. Ac-Phe was coupled with *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB) in the presence of *N,N'*-dicyclohexylcarbodiimide (Fujino et al., 1974). The active ester of Ac-Phe (1 mmol) was added to tetrahydrofuran solution (4 mL) of Phe-Ado (0.2 mmol), and the reaction mixture was stirred for 30 min at 0 °C and then overnight at room temperature. The solution was evaporated to dryness in vacuo, and the residue was chromatographed on a silica gel column (20 mL) by stepwise elution with chloroform containing 5–10% (v/v) methanol. The eluate was checked by silica gel thin-layer chromatography in the system chloroform–methanol (9:1). The UV-absorbing fraction ($R_f$ = 0.4) was positive in chlorine–tolidine reaction, and the UV spectrum (220–300 nm) of this fraction in methanol was similar to that of adenosine. The reaction product was in fact identified as Ac-Phe-Phe-Ado by analysis of the 270-MHz proton NMR spectrum, on the basis of the proton resonance assignments previously made for Phe-Ado (Taiji et al., 1981, 1983).

*Preparation of N-Acetyl[$^3$H]phenylalanyl-$tRNA^{Phe}$.* *E. coli* $tRNA^{Phe}$ was charged with L-[$^3$H]phenylalanine by phenylalanyl-tRNA synthetase from *T. thermophilus* HB8. The reaction was carried out in a mixture containing 100 mM Tris-HCl (pH 7.5), 10 mM KCl, 5 mM magnesium acetate, 2 mM ATP, 0.8 $\mu$M L-phenyl[2,3-$^3$H]alanine (3860 Ci/mol), 0.2 $A_{260}$ units/mL *E. coli* $tRNA^{Phe}$, and 10 units/mL phenylalanyl-tRNA synthetase.[2] The reaction mixture was incubated for 15 min at 65 °C, and thus the extent of aminoacylation of $tRNA^{Phe}$ was raised higher than 60%. The phenylalanyl residue of [$^3$H]phenylalanyl-$tRNA^{Phe}$ was N-acetylated nearly completely by treatment with acetic anhydride (Haenni & Chapeville, 1966). The product, *N*-acetyl[$^3$H]phenylalanyl-$tRNA^{Phe}$ (Ac-[$^3$H]Phe-$tRNA^{Phe}$) was dissolved in 20 mM potassium acetate buffer (pH 5.0) and stored at −80 °C.

*Peptidyltransferase Reaction.* Nonenzymatic binding of Ac-[$^3$H]Phe-$tRNA^{Phe}$ to the ribosomal P site was performed in a reaction mixture (250 $\mu$L) containing 50 mM Bes–KOH buffer (pH 7.0 at 0 °C), 150 mM ammonium chloride, 10 mM 2-mercaptoethanol, 10 mM magnesium acetate, 50 $\mu$g of poly(U), 80 pmol of Ac-[$^3$H]Phe-$tRNA^{Phe}$, and 6.7 $A_{260}$ units of *E. coli* 70S ribosome. This reaction mixture was preincubated for 10 min at 37 °C, in order to allow the formation

[2] For aminoacyl-tRNA synthetases (EC 6.1.1) from *T. thermophilus*, the enzyme unit has been defined (Hara-Yokoyama et al., 1984).

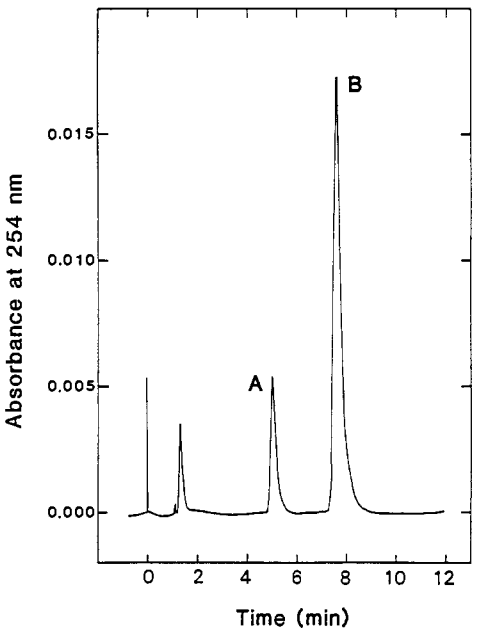


FIGURE 2: HPLC elution profile of Ac-Phe-Phe-Ado, as chemically synthesized, on an ODS column at 28 °C. Peaks A and B are due to the 2′-isomer and 3′-isomer, respectively.

of the Ac-[$^3$H]Phe-tRNA$^{Phe}$·poly(U)·ribosome complex and to reactivate the peptidyltransferase (Miskin et al., 1968). Nitrocellulose membrane filter assay indicated that more than 75% of Ac-[$^3$H]Phe-tRNA$^{Phe}$ was bound to the ribosome after the preincubation. The reaction mixture was then chilled on ice, and the peptidyltransferase reaction was initiated by the addition of Phe-Ado to 1 mM. The reaction was allowed to proceed for 1–60 min at 0 °C and then was terminated by the addition of 8.5 M acetic acid. The reaction product was extracted in ethyl acetate and was evaporated to dryness in vacuo. The residue was dissolved in 1 mM sodium phosphate buffer (pH 2.2) and analyzed by HPLC on an ODS column, where isocratic elution was performed with 5 mM sodium phosphate buffer containing 50% (v/v) methanol and was monitored with $^3$H radioactivity.

## Results

*Separation of 2′-Isomer and 3′-Isomer of Ac-Phe-Phe-Ado.* The proton NMR spectrum of Ac-Phe-Phe-Ado as chemically synthesized was analyzed, and in [$^2H_4$]methanol solution at 23 °C, the abundance ratio of the 2′-isomer and 3′-isomer was found to be 0.19:0.81, from the intensity comparison of the proton resonances of the two isomers. For the chemical separation of the two isomers, a variety of experimental conditions for HPLC were tested in comparison (to be discussed later). Finally, a suitable condition was found for clear separation of the 2′-isomer and 3′-isomer of Ac-Phe-Phe-Ado. 0.01 $A_{260}$ unit of Ac-Phe-Phe-Ado as chemically synthesized was dissolved in methanol and applied to an ODS column. Isocratic elution was performed with 5 mM sodium phosphate buffer (pH 2.5) containing 50% (v/v) methanol. In the elution profile, two major peaks (isomer A and isomer B) were observed, as shown in Figure 2. The area intensity ratio of these two peaks was found to be 0.20:0.80, closely corresponding to the abundance ratio (0.19:0.81) of the 2′-isomer and 3′-isomer as determined by proton NMR analysis. The UV spectra of isomers A and B were both found to be nearly the same as that of the isomer mixture of Ac-Phe-Phe-Ado as chemically synthesized. Fractions of isomer A and isomer B were collected and extracted with chloroform. The chloroform–methanol phase was evaporated to dryness in vacuo,

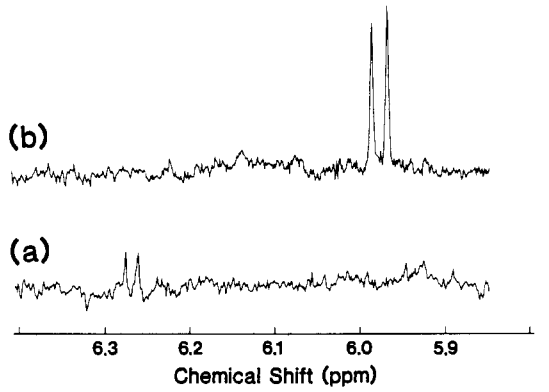


FIGURE 3: 400-MHz proton NMR spectra (6.3–5.9 ppm) of isomer A (50 μM) (a) and isomer B (200 μM) (b) of Ac-Phe-Phe-Ado in [$^2H_4$]methanol solution at 23 °C. Because of slow transacylation, the H1′ proton resonance of only one isomer is observed in each spectrum.

dissolved in 1 mM sodium phosphate buffer (pH 2.2), and again analyzed by HPLC. Thus, isomers A and B were both found to be pure and cleanly separated from each other.

In order to analyze the chemical structures of the two isomers, the 400-MHz proton NMR spectra of isomers A and B as isolated by HPLC were measured in [$^2H_4$]methanol solution (spectra a and b of Figure 3). The ribose H1′ proton resonances of isomers A and B were observed at 6.27 and 5.98 ppm, respectively. For a variety of aminoacyladenosines, the H1′ proton resonance of the 2′-isomer has been observed downfield (by 0.20–0.45 ppm) as compared with that of the 3′-isomer (Taiji et al., 1981, 1983). Therefore, isomers A and B of Ac-Phe-Phe-Ado are now identified as the 2′-isomer and 3′-isomer, respectively. These two isomers of Ac-Phe-Phe-Ado are clearly separated from each other by the HPLC method, and each purified isomer can be stored in ethyl acetate, chloroform–methanol (9:1), or 1 mM sodium phosphate buffer (pH 2.2) at −80 °C for several weeks without transacylation.

*2′ ⇌ 3′ Equilibration Rate of Ac-Phe-Phe-Ado As Determined by HPLC Method.* The transacylation between the 2′-isomer and 3′-isomer of Ac-Phe-Phe-Ado in aqueous solution at pH 7.0 and 0 °C was followed by HPLC analysis on an ODS column. The equilibration of Ac-Phe-Phe-Ado was initiated by dissolving the 2′-isomer (in a small volume of 1 mM sodium phosphate buffer at pH 2.5) in 500 mM Bes–KOH buffer (pH 7.0 at 0 °C). After incubation at 0 °C for a given duration of time, the equilibration reaction was quenched by the addition of 8.5 M acetic acid. The isomer mixture of Ac-Phe-Phe-Ado was extracted in ethyl acetate, and the ethyl acetate phase was evaporated in vacuo. The residue was dissolved in 1 mM sodium phosphate buffer (pH 2.2) and analyzed by HPLC on an ODS column, where an isocratic elution was performed with 5 mM sodium phosphate buffer (pH 2.5) containing 60% (v/v) methanol. The abundance ratio of the 2′-isomer and 3′-isomer was obtained from the ratio of the area intensities of the peaks in the elution profiles. After incubation for 10 min, the isomer equilibrium was reached and the abundance ratio of the 2′-isomer and 3′-isomer was found to be 0.30:0.70 at pH 7.0 and 0 °C.

The 2′ ⇌ 3′ equilibration rate $k(2' \rightleftharpoons 3')$ is equal to the sum of the 2′ → 3′ transacylation rate $[k(2' \rightarrow 3')]$ and the 3′ → 2′ transacylation rate $[k(3' \rightarrow 2')]$. The fractional population $[f(t)]$ of one isomer (2′-isomer or 3′-isomer) after incubation for time $t$ is given by

$$\ln \{[f(\infty) - f(0)]/[f(\infty) - f(t)]\} = kt \qquad (1)$$

For the equilibration process starting from the 2′-isomer, the

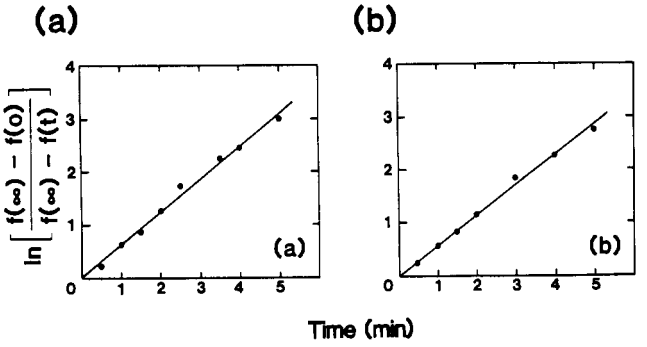


FIGURE 4: Plot of $\ln\{[f(\infty)-f(0)]/[f(\infty)-f(t)]\}$ against the incubation time ($t$) for the $2' \rightleftharpoons 3'$ equilibration process of Ac-Phe-Phe-Ado, starting from the 2'-isomer (a) or from the 3'-isomer (b).

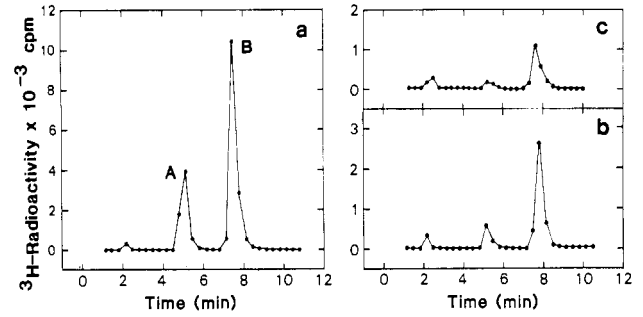


FIGURE 5: HPLC elution profile of Ac-[$^3$H]Phe-Phe-Ado (peak A, 2'-isomer; peak B, 3'-isomer), as the product of the peptidyltransferase reaction after incubation for 60 min (a), 3 min (b), or 1 min (c). The small peak eluted at 2 min is due to Ac-[$^3$H]Phe as formed by the deacylation of Ac-[$^3$H]Phe-tRNA$^{Phe}$.

plot of $\ln\{[f(\infty)-f(0)]/[f(\infty)-f(t)]\}$ against time $t$ is shown in Figure 4a. From the slope of the straight line as obtained by the method of least squares, the rate constant $k(2' \rightleftharpoons 3')$ was found to be $0.62 \pm 0.03$ min$^{-1}$. Conversely, for the equilibration process starting from the 3'-isomer (Figure 4b), the rate constant $k(2' \rightleftharpoons 3')$ was obtained as $0.57 \pm 0.02$ min$^{-1}$. From these values, the $2' \rightleftharpoons 3'$ equilibration rate of Ac-Phe-Phe-Ado at pH 7.0 at 0 °C was thus found to be $0.59 \pm 0.04$ min$^{-1}$. Then, the transacylation rates may be obtained by use of the equilibration rate and the abundance ratio of the two isomers (Taiji et al., 1983). Thus, the transacylation rates of Ac-Phe-Phe-Ado were obtained as $k(2' \rightarrow 3') = 0.41$ min$^{-1}$ and $k(3' \rightarrow 2') = 0.18$ min$^{-1}$.

*Abundance Ratio of 2'-Isomer and 3'-Isomer of Ac-[$^3$H]-Phe-Phe-Ado As Formed by Peptidyltransferase Reaction.* The HPLC method was then used for analysis of the 2'/3'-isomer specificity in the peptidyltransferase reaction with *isomerizable* acceptor Phe-Ado. As described under Experimental Procedures, Phe-Ado was used as the acceptor of the Ac-[$^3$H]Phe group in the Ac-[$^3$H]Phe-tRNA$^{Phe}$·poly(U)·ribosome system. After the reaction was allowed to proceed for 60 min at pH 7.0 and 0 °C, the reaction products were analyzed by HPLC on an ODS column (Figure 5a) and were in fact identified as the 2'-isomer and 3'-isomer of Ac-[$^3$H]-Phe-Phe-Ado. The abundance ratio of the 2'-isomer and 3'-isomer was obtained as 0.30:0.70 from the integrated $^3$H radioactivities of the corresponding peaks in the elution profile. However, the ultracentrifugation analysis (data not shown) indicated that the reaction product, Ac-[$^3$H]Phe-Phe-Ado, was largely released from the ribosome after the peptidyltransferase reaction. Then, the $2' \rightleftharpoons 3'$ equilibration of free Ac-[$^3$H]-Phe-Phe-Ado should be attained during the incubation of the reaction solution for 60 min. The abundance ratio (0.30:0.70) of the two isomers of Ac-[$^3$H]Phe-Phe-Ado was in fact equal to that obtained for the equilibrium mixture of the two isomers of the authentic preparation of Ac-Phe-Phe-Ado at pH 7.0 and 0 °C (as determined by UV absorption).

By contrast, upon termination of the peptidyltransferase reaction after a short incubation time, the fractional population of the 2'-isomer was much lower than the equilibrium population of 30%. Thus, the abundance ratio of the 2'-isomer and 3'-isomer was 0.17:0.83 after incubation for 3 min (Figure 5b) and 0.13:0.87 after incubation for 1 min (Figure 5c). From extrapolation to zero incubation time, the initial product of the peptidyltransferase reaction was found to be exclusively the 3'-isomer, rather than the 2'-isomer, of Ac-[$^3$H]Phe-Phe-Ado.

## Discussion

*Separation of 2'-Isomer and 3'-Isomer of Peptidyladenosine by HPLC Method.* In our previous study on aminoacyladenosines, we have analyzed the pH dependences of the transacylation rates in aqueous solution and found that the transacylation reactions are base catalyzed (Taiji et al., 1983). This suggests that the transacylation rates of aminoacyladenosine will be significantly reduced upon acylation of the $\alpha$-amino group of aminoacyladenosines. In fact, the 2'-isomer and 3'-isomer of *N*-benzyloxycarbonylcycloleucyladenosine have been separated by HPLC on a silica gel column (Chládek & Bhuta, 1982).

The development of an HPLC method for the clear separation of the two isomers of peptidyladenosines will lead to a methodology for studying the 2'/3'-isomer specificity of native (isomerizable) aminoacyl-tRNA and peptidyl-tRNA species in the polypeptide chain elongation cycle. Accordingly, in this study, we have undertaken the separation of the two isomers of peptidyladenosine. In view of the base-catalyzed scheme of the transacylation of the two isomers, we have chosen an acidic buffer (sodium phosphate buffer at pH 2.5) for isocratic elution. Furthermore, for better separation of the two isomers of Ac-Phe-Phe-Ado, we have used an ODS column and added 50–60% (v/v) methanol to the elution buffer, taking into account the hydrophobic nature of the two phenylalanine residues of Ac-Phe-Phe-Ado. Thus, we have succeeded in clear separation of the 2'-isomer and 3'-isomer of Ac-Phe-Phe-Ado. With this HPLC method, the difference in retention time between the 2'-isomer and 3'-isomer is sufficiently large (as large as 3 min) for the determination of the abundance ratio and for the isolation of each isomer without contamination by the other isomer.

Each of the two isomers of Ac-Phe-Phe-Ado may be efficiently extracted in chloroform from the eluate, and the equilibration between the two isomers is negligibly slow in organic solvent and in acidic aqueous solution (sodium phosphate buffer at pH 2.5). The equilibration rate of Ac-Phe-Phe-Ado is slow even in neutral aqueous solution (sodium phosphate buffer at pH 7.0) at 0 °C; $k(2' \rightleftharpoons 3')$ is as slow as 0.6 min$^{-1}$. On the other hand, the equilibration rate of Phe-Ado in aqueous solution at pH 7.0 and 0 °C was estimated to be $k(2' \rightleftharpoons 3') = 8$ min$^{-1}$ from the values of the rate constants and the enthalpy of activation as obtained previously (Taiji et al., 1983). Phe-Ado has already been found to be a good model for the CCA-terminal moiety of aminoacyl-tRNA, since, for example, the 5'-phosphate group does not affect transacylation rate (Taiji et al., 1983). Similarly, Ac-Phe-Phe-Ado may be regarded as a good model for peptidyl-tRNA. Therefore, the transacylation rates of peptidyl-tRNA are expected to be even slower than those of aminoacyl-tRNA.

*Analysis of 2'/3'-Isomer Specificity of Peptidyltransferase Reaction by HPLC Method.* The 2'/3'-isomer specificity for

the peptide acceptor at the A site of ribosome has been studied by the use of nonisomerizable deoxy analogues such as 2′-aminoacyl-3′-deoxy-Ado oligonucleotides and 3′-aminoacyl-2′-deoxy-Ado oligonucleotides (Hussain & Ofengand, 1973; Chládek et al., 1973, 1974; Chládek, 1980; Bhuta et al., 1981). Then for the study of the 2′/3′-isomer specificities for aminoacyl-tRNA and peptidyl-tRNA in the polypeptide chain elongation cycle, nonisomerizable tRNA analogues, including 2′-deoxy-Ado-tRNA and 3′-deoxy-Ado-tRNA, have been used (Hecht, 1979; Sprinzl & Wagner, 1979; Wagner et al., 1982; Wagner & Sprinzl, 1983). Thus, in the A site of ribosome, 3′-aminoacyl-2′-deoxy-Ado-tRNA (rather than 2′-aminoacyl-3′-deoxy-Ado-tRNA) has been found to accept the nascent peptide efficiently from the P site (Hecht et al., 1974; Sprinzl & Wagner, 1979; Wagner et al., 1982). However, 3′-peptidyl-2′-deoxy-Ado-tRNA thus formed in the A site cannot be translocated to the P site even with polypeptide chain elongation factor G (EF-G) (Wagner & Sprinzl, 1983). This suggests that the 2′-OH group of the CCA-terminal adenosine residue of peptidyl-tRNA is required for the EF-G-dependent translocation. Furthermore, as long as nonisomerizable tRNA analogues are used, it is not possible to discover which isomer (2′ or 3′) of peptidyl-tRNA is formed after the peptidyl transfer to native (isomerizable) aminoacyl-tRNA in the A site.

In this present study, we have developed a useful HPLC method for the analysis of the abundance ratio of the 2′-isomer and 3′-isomer of peptidyladenosine. Therefore, it is now possible to use isomerizable tRNA analogues for elucidating the 2′/3′-isomer specificities in the peptidyltransferase reaction, by combination of the in vitro assay system with this HPLC method. Immediately after the peptidyl transfer from Ac-[$^3$H]Phe-tRNA$^{Phe}$ in the P site, the 3′-isomer (rather than the 2′-isomer) of Ac-[$^3$H]Phe-Phe-Ado has been found to be the reaction product by the HPLC method as monitored with $^3$H radioactivity (Figure 5c). This clearly indicates the specificity for the 3′-isomer of aminoacyladenosine as the peptide acceptor in the peptidyltransferase center.

It should be remarked, however, that the binding affinities of aminoacyladenosine and peptidyladenosine to the ribosome are much weaker than those of aminoacyl-tRNA and peptidyl-tRNA, respectively. Therefore, it will be important to elucidate the 2′/3′-isomer specificity of the peptidyltransferase reaction, with native (isomerizable) aminoacyl-tRNA species (rather than aminoacyladenosine) in the A site of ribosome. We have already found that ribonucleases are useful for cleaving peptidyladenosine from peptidyl-tRNA as formed in the A site. Thus, the analyses of the 2′/3′-isomer specificity in the polypeptide chain elongation cycle are now in progress in our laboratory (to be reported separately), on the basis of the HPLC method as reported here for the analysis of the abundance ratio of the 2′-isomer and 3′-isomer of peptidyladenosine.

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