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Aminoacyl Transfer Ribonucleic Acid Binding Site of the Bacterial Elongation Factor Tu†

Alfred Pingoud* and Claus Urbanke

ABSTRACT: Hydrolysis protection experiments were used for a quantitative determination of the binding of several aminoacyl-tRNAs to the *Escherichia coli* elongation factor Tu. The observed differences could not be rationalized in terms of structural properties of the tRNAs. The experimental results support, however, a model according to which the differences in the affinity of naturally occurring aminoacyl-tRNAs are determined mainly by the nature of the amino acid esterified to the tRNA. Aminoacyl-tRNAs with polar amino acid side chains are bound less strongly than those with apolar ones. This model is substantiated by results obtained with misacylated and modified aminoacyl-tRNAs. Furthermore, it could be shown that the aminoacyl group of the aminoacyl-tRNA must be in the L configuration; EF-Tu in this way prevents blocking of the ribosomal A site or even incorporation of D-amino acids into protein. The data have been used for a schematic description of the structure of a part of the aminoacyl-tRNA binding site of the bacterial elongation factor Tu.

Elongation factor (EF-Tu)[1] supplies the bacterial ribosome with aminoacyl-tRNAs (aa-tRNAs) [for recent reviews, cf. Lucas-Lenard & Beres (1974), Miller & Weissbach (1977), and Ofengand (1977)]. For this purpose a ternary complex consisting of one molecule each of EF-Tu·GTP and aa-tRNA is formed. The structural requirements of both the nucleotide and the tRNA for ternary complex formation have been the subject of several investigations.

The involvement of the acceptor and TΨCG stems of the tRNA in the aa-tRNA·EF-Tu·GTP complex formation has been demonstrated by Jekowsky et al. (1977) in nuclease digestion experiments. The importance of contributions from the end of the acceptor stem to ternary complex formation has been deduced from experiments with several aa-tRNAs lacking the 5′-terminal phosphate. These aa-tRNAs are drastically impaired in their ability to form stable ternary complexes (Schulman et al., 1974). Factor-dependent binding to the ribosome is diminished by a replacement of the terminal adenosine by formycin (Baksht et al., 1975) and by lengthening the CCA terminus by introducing another CMP residue (Thang et al., 1972). Furthermore, several aminoacylated single-stranded fragments of aa-tRNAs have been demonstrated not to bind to EF-Tu·GTP; i.e., within the concentration ranges investigated strong complex formation with $K_{assoc} > 10^5$ M^{-1} could be excluded (Kawakami et al., 1975; Krausskopf et al., 1972). This finding, however, is not necessarily in disagreement with the reported binding of smaller fragments, e.g., CpA-Phe. In these experiments high concentrations of the aminoacyl dinucleoside phosphate had been employed; binding constants of the order of 10^5 M^{-1} had been deduced (Ringer & Chladek, 1975; Jonak et al., 1978). With respect to the aminoacyl moiety, it had been shown that *N*-acyl-aminoacyl-tRNAs are not bound by EF-Tu·GTP (Ravel et al., 1967; Weissbach et al., 1978). Hydroxyacyl-tRNAs, however, are bound (Fahnenstock et al., 1972). Similarly, modifications in the 3′-terminal nucleotide affect ternary complex formation: Phe-tRNA(Phe, yeast)(ox–red) does not form a complex with EF-Tu·GTP (Ofengand & Chen 1972); Tyr-tRNA(Tyr, yeast) lacking the vicinal OH group either in the 2′ or 3′ position is bound weakly to EF-Tu·GTP, and Phe-tRNA(Phe, yeast) with a 2′- or 3′-NH_2 group carrying the aminoacyl residue does not interact with EF-Tu·GTP (Sprinzl et al., 1977).

There are considerable differences in the association constants for the binding of some naturally occurring tRNAs to EF-Tu·GTP (Pingoud et al., 1977). We have, therefore, extended this investigation by analyzing in precise physicochemical terms the binding of several other aa-tRNAs, unmodified and modified ones; this allows us now to put forward

† From the Abteilung Biophysikalische Chemie, Zentrum Biochemie, Medizinische Hochschule, D-3000 Hannover, West Germany. *Received August 22, 1978; revised manuscript received December 27, 1979.* The work was supported by a grant from the Deutsche Forschungstgemeinschaft (Schwerpunktprogramm Molekulare Biologie). A preliminary account of this work had been presented at the 12th FEBS Meeting, Dresden, 1978, and the 11th International Congress of Biochemistry, Toronto, 1979.

[1] Abbreviations used: EF-Tu, elongation factor Tu from *E. coli*; aa-tRNA, aminoacyl transfer ribonucleic acid; Tyr-tRNA (Tyr, *E. coli*), tyrosine-specific tyrosyl-tRNA from *E. coli* (other aminoacyl-tRNAs are abbreviated analogously); p-F-Phe-tRNA (Phe, yeast), tRNA(Phe, yeast) aminoacylated with *p*-fluorophenylalanine; Tyr-tRNA(Tyr, yeast)(3′-dA), Tyr-tRNA(Tyr, yeast) lacking the 3′-OH group at the terminal adenosine [analogously for Tyr-tRNA(Tyr, yeast)(2′-dA)]; Phe-tRNA(Phe, yeast)(CCF), Phe-tRNA(Phe, yeast) with the terminal adenosine replaced by formycin; Phe-tRNA(Phe, yeast)(ox–red) is Phe-tRNA(Phe, yeast) with the ribose ring of the terminal adenosine cleaved by periodate oxidation with a subsequent $NaBH_4$ reduction; Tris, tris(hydroxymethyl)aminomethane.

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a plausible explanation for the differences in the binding of aa-tRNAs to EF-Tu·GTP and to make a reasonable proposition for the structural features of a part of the aminoacyl-tRNA binding site of EF-Tu.

Materials and Methods

EF-Tu·GTP was isolated from *Escherichia coli* MRE 600 as described recently (Pingoud et al., 1977). It had a GDP-binding capacity of over 30 nmol of GDP per $A_{280\ nm}$ unit. Phenylalanyl-tRNA synthetase from *E. coli* and yeast were generously provided by Dr. G. Krauss, tRNA(Tyr, *E. coli*) was prepared as reported previously (Pingoud et al., 1975), and tRNA (Trp, *E. coli*) was prepared from crude tRNA by using BD-cellulose and RPC-5 chromatography. It had an amino acid acceptance of 1200 nmol/$A_{260\ nm}$ unit. tRNA(Tyr, yeast) was enriched starting from crude yeast tRNA by using BD-cellulose and Sepharose 4B chromatography (Goppelt, 1976). It had an amino acid acceptance of 640 nmol/$A_{260\ nm}$ unit. tRNA(Phe, *E. coli*), tRNA(Phe, yeast), tRNA(Lys, *E. coli*), tRNA(Glu, *E. coli*), and crude tRNA from yeast and *E. coli* were purchased from Boehringer (Mannheim). Pyruvate kinase, phosphoenolpyruvate, GTP, ATP, and 1,4-dithioerythritol were obtained from Boehringer (Mannheim); D-tyrosin was purchased from Serva (Heidelberg). All other reagents were obtained from Merck (Darmstadt). L-(^{14}C)-tyrosine, L-(^{14}C)phenylalanine, L-(^{3}H)tryptophane, (^{3}H)-*p*-F-phenylalanine, L-(^{14}C)glutamic acid, and L-(^{14}C)lysine were purchased from Amersham Buchler (Braunschweig); D-(^{3}H)tyrosine was obtained from New England Nuclear (Dreieich).

Radioactively labeled aminoacyl-tRNAs were prepared as described recently (Pingoud et al., 1977). (^{14}C)Tyr-tRNA-(Tyr, *E. coli*), (^{14}C)Phe-tRNA(Phe, yeast), (^{14}C)Phe-tRNA-(Phe, *E. coli*), (^{3}H)-p-F-Phe-tRNA(Phe, yeast), and (^{3}H)-Trp-tRNA(Trp, *E. coli*) were obtained by incubating 15A_{260nm} units of the tRNA for 5 min at 37 °C in 5 mL of 20 mM potassium phosphate, pH 7.5, 25 mM $MgCl_2$, 0.5 mM 1.4-dithioerythritol, 5 mM ATP, 10 μM amino acid, and sufficient amounts of the cognate synthetase so as to ensure maximum charging within 5 min. For the preparation of (^{3}H)Trp-tRNA(Trp, *E. coli*), an aminoacyl-tRNA synthetase preparation from *E. coli* enriched in tryptophanyl-tRNA synthetase was used. (^{14}C)Lys-tRNA(Lys, *E. coli*) was prepared by incubating 15 $A_{260\ nm}$ units of tRNA(Lys, *E. coli*) at 37 °C for 5 min in 5 mL of 30 mM potassium phosphate, pH 7.5, 50 mM KCl, 1 mM $MgCl_2$, 2 mM ATP, 0.5 mM 1,4-dithioerythritol, 1 mM spermine, 10 μM L-(^{14}C)lysine, and an adequate amount of a lysyl-tRNA synthetase preparation from *E. coli* (^{14}C)Phe-tRNA(Lys, *E. coli*) was obtained by incubating 10 $A_{260\ nm}$ units of tRNA(Lys, *E. coli*) at 37 °C for 10 min in 10 mL of 10 mM Tris-HCl, pH 8.5, 8 mM $MgSO_4$, 0.5 mM ATP, 30 μM L-(^{14}C)phenylalanine, and 10 μM phenylalanyl-tRNA synthetase from yeast. (^{14}C)Glu-tRNA-(Glu, *E. coli*) was prepared by incubation of 10 $A_{260\ nm}$ units of tRNA(Glu, *E. coli*) for 15 min at 37 °C in 10 mL of 200 mM sodium cacodylate, pH 7.2, 20 mM $MgCl_2$, 20 mM KCl, 0.4 mM ATP, 1 μM L-(^{14}C)glutamic acid, and a sufficient amount of a glutamyl-tRNA synthetase preparation from *E. coli* [supplied by Dr. Loewen, Boehringer (Mannheim)]. All tRNAs mentioned above have been aminoacylated to an extent of more than 60%. tRNA(Tyr, yeast) was aminoacylated in a mixture of tRNAs from yeast enriched in tRNA(Tyr). The aminoacylation reaction was carried out by incubating 200 A_{260nm} units of tRNA at 37 °C for 5 min in 5 mL of 150 mM Tris-HCl, pH 7.6, 50 mM KCl, 10 mM $MgCl_2$, 2 mM ATP, 0.5 mM 1,4-dithioerythritol, 10 μM L-(^{14}C)tyrosine, and a sufficient amount of a tyrosyl-tRNA synthetase preparation (Goppelt, 1976). In this preparation 5% of the total tRNA was aminoacylated with (^{14}C)tyrosine. As indicated below, the presence of uncharged tRNAs is of no consequence for the performance of hydrolysis protection experiments, which can be carried out even with crude tRNA mixtures, in which, however, only one tRNA species must be charged specifically with one amino acid. D-Tyr-tRNA(Tyr, *E. coli*) was prepared by adaptation of the assay procedure of Calendar & Berg (1966) using D-tyrosine and radioactively labeled D-tyrosine. Under the conditions used, tRNA(Tyr, *E. coli*) was charged to an extent of 15% as compared to the reaction with the L isomer. All aminoacyl-tRNAs were purified by extraction with phenol and subsequently desalted over Sephadex G-25 fine (Pharmacia). The concentrations of radioactively labeled aa-tRNAs were determined by liquid scintillation counting with amino acids of the same specific activity for reference.

Hydrolysis protection experiments were carried out as reported in detail recently (Pingoud et al., 1977; Pingoud & Urbanke, 1978). A typical experiment and the detailed procedure is given in Figure 1. In these experiments advantage is taken of the protective effect of ternary complex formation on the stability of the aminoacyl bond against nonenzymatic hydrolysis. For the quantitative description of the net hydrolysis, three reactions have to be considered:

$$\text{aa-tRNA} \xrightarrow{k_{free}} \text{amino acid} + \text{tRNA} \quad (1)$$

$$\text{aa-tRNA·EF-Tu·GTP} \xrightarrow{k_{bound}} \text{amino acid} + \text{tRNA} + \text{EF-Tu·GTP} \quad (2)$$

$$\text{aa-tRNA} + \text{EF-Tu·GTP} \underset{}{\overset{K_{assoc}}{\rightleftharpoons}} \text{aa-tRNA·EF-Tu·GTP} \quad (3)$$

Reaction 3 is considered to be fast compared to reactions 1 and 2. The time course of hydrolysis is then given by the equations

$$\frac{d([\text{aa-tRNA}] + [\text{aa-tRNA·EF-Tu·GTP}])}{dt} = k_{free}[\text{aa-tRNA}] + k_{bound}[\text{aa-tRNA·EF-Tu·GTP}] \quad (4)$$

$$K_{assoc} = \frac{[\text{aa-tRNA·EF-Tu·GTP}]}{[\text{aa-tRNA}][\text{EF-Tu·GTP}]} \quad (5)$$

The hydrolysis progress curves are evaluated by a fitting procedure using an analog computer, which yields the binding constant K_{assoc} for complex formation between EF-Tu·GTP and aa-tRNA as well as the rate constants of hydrolysis of free and complex-bound aa-tRNA, k_{free} and k_{bound}. This evaluation implies that a direct comparison of individual experiments with different aa-tRNAs is only meaningful if k_{free}, k_{bound}, and the initial concentrations of EF-Tu·GTP and aa-tRNAs are identical. For the determination of binding constants, usually more than five hydrolysis protection experiments with different initial concentrations of aa-tRNA and EF-Tu·GTP were carried out.

For a graphical representation of all experiments, a plot is employed which formally corresponds to a Scatchard plot (Scatchard, 1949; Pingoud et al., 1977). By use of the value of K_{assoc} determined by the fitting procedure (see above), the theoretical ratio of free and complex-bound aa-tRNA is calculated. This ratio and the experimentally determined total aa-tRNA concentration are used to calculate ν = [aa-tRNA·EF-Tu·GTP]/[EF-Tu·GTP]$_{total}$ and ν/[aa-tRNA]$_{free}$.

Hydrolysis protection experiments are undisturbed by the presence of uncharged tRNAs, since these tRNAs have been shown to be bound by EF-Tu·GTP by a factor of 10^3–10^4 more

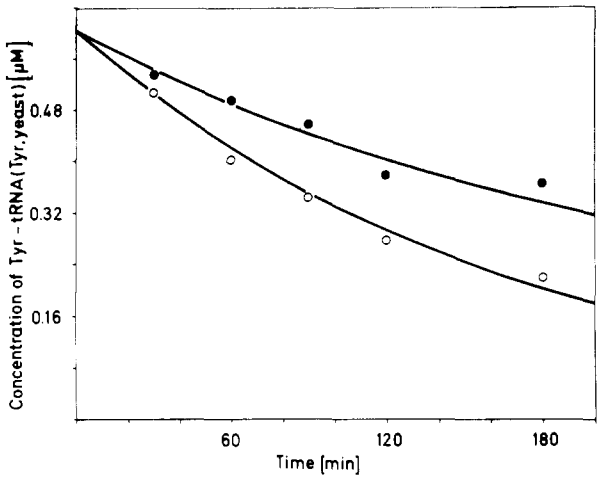

FIGURE 1: Hydrolysis protection experiments with Tyr-tRNA(Tyr, yeast) at 25 °C. 0.6 μM (^{14}C)Tyr-tRNA(Tyr, yeast) was incubated without (○) and with 0.36 μM EF-Tu (●) in 75 mM Tris-HCl, pH 7.4, 75 mM NH_4Cl, 15 mM $MgCl_2$, 7.5 mM 1,4-dithioerythritol, 2.25 mM phosphoenolpyruvate, 0.1 mM GTP, and 0.15 mg/mL pyruvate kinase. At the times indicated, 20-μL aliquots were applied to Whatman 3MM filter disks, which were immersed in cold 10% w/v trichloroacetic acid, and washed with 5% w/v trichloroacetic acid, then with an ethanol–ether mixture (1:1 v/v), and finally with ether. The dried filter disks were assayed for radioactivity in a liquid scintillation spectrometer. The drawn out lines are the simulated hydrolysis progress curves for the hydrolysis of free aminoacyl-tRNA (lower curve) and of the aminoacyl-tRNA in the presence of EF-Tu·GTP assuming a K_{assoc} of 4×10^6 M^{-1} and $k_{free}/k_{bound} = 50$. These values have been determined from a set of experiments with different initial concentrations of aa-tRNA and EF-Tu·GTP.

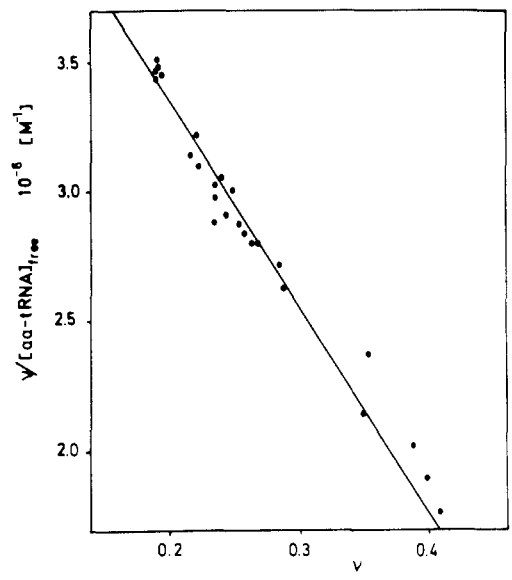

FIGURE 2: Hydrolysis protection experiments with Tyr-tRNA(Tyr, yeast) at 25 °C represented as a Scatchard-type plot. For details of the construction of the plot cf. Materials and Methods.

weakly (Gordon, 1967; Shulman et al., 1974) and therefore cannot interfere with hydrolysis protection experiments. We have verified that a 100-fold excess of uncharged tRNA(Tyr, *E. coli*) does not change the hydrolysis protection of Tyr-tRNA(Tyr, *E. coli*) by EF-Tu·GTP.

Results and Discussion

We had established previously that aa-tRNAs differ in their affinities toward EF-Tu·GTP, Phe-tRNA(Phe, yeast) binding nearly 10 times more strongly than Tyr-tRNA(Tyr, *E. coli*) and Ser-tRNA(Ser, yeast). As a working hypothesis we had assumed that this may be due to the fact that both Ser-tRNA(Ser, yeast) and Tyr-tRNA(Tyr, *E coli*) contain a large variable arm which might interfere with strong binding. A series of hydrolysis protection experiments with Tyr-tRNA-(Tyr, yeast) which does not contain a large variable arm showed that this Tyr-tRNA(Tyr) binds with an almost identical affinity to EF-Tu·GTP as Tyr-tRNA(Tyr, *E. coli*) and Ser-tRNA(Ser, yeast) [Figures 1 and 2 and Pingoud et al. (1977)]. This indicates that it is probably not the size of the variable arm that determines the differences in the binding constant. This result is in agreement with the finding that the variable arm of the tRNA is not protected against nuclease digestion by ternary complex formation (Jekowsky et al., 1977) as well as with experiments that show that the quantum yield of fluorescent reporter groups like fluorescamine introduced into this part of Phe-tRNA(Phe, *E. coli*) is not changed by the presence of EF-Tu·GTP (Sprinzl & Faulhammer, 1978).

Since the acceptor stem is involved in ternary complex formation, we checked whether the base composition had an effect on the stability of the ternary complex. A comparison of the binding of Phe-tRNA(Phe, yeast) (Pingoud et al., 1977) and Phe-tRNA(Phe, *E. coli*) which have a considerably different G·C content in the acceptor stem shows that these Phe-tRNA(Phe)'s are bound equally well within the limits of

Table I: Binding Constants for Complex Formation between EF-Tu·GTP and Aminoacyl-tRNAs[a]

aminoacyl-tRNA	$K_{assoc} \times 10^{-6}$
Trp-tRNA(Trp, *E. coli*)	50
Phe-tRNA(Phe, *E. coli*)	50
Phe-tRNA(Phe, yeast)	50
Phe-tRNA(Lys, *E. coli*)	20
p-F-Phe-tRNA(Phe, yeast)	10
Tyr-tRNA(Tyr, *E. coli*)	7
Ser-tRNA(Ser, yeast)	5
Glu-tRNA(Glu, *E. coli*)	4
Lys-tRNA(Lys, *E. coli*)	4
Tyr-tRNA(Tyr, yeast)	4
Tyr-tRNA(Tyr, yeast)(3′-dA)	3
Tyr-tRNA(Tyr, yeast)(2′-dA)	0.5
Phe-tRNA(Phe, yeast)(CCF)	0.5
D-Tyr-tRNA(Tyr, *E. coli*)	<0.1[b]

[a] The binding constants for 2′- and 3′-dA tRNAs were determined at 37 °C. The constants at 25 °C can be estimated to be about twofold higher. [b] No binding detected.

error, suggesting that the affinities of different aa-tRNAs to EF-Tu·GTP might be influenced by the aminoacyl residue, i.e., that they will be similar if the amino acid side chain of the aa-tRNA is the same (cf. Table I).

In order to demonstrate that the aminoacyl group is at least in part responsible for the observed differences in affinity of various aminoacyl-tRNAs toward EF-Tu·GTP, we have measured the binding of two mischarged aminoacyl-tRNAs to EF-Tu·GTP: p-F-Phe-tRNA(Phe, yeast) and Phe-tRNA-(Lys, *E. coli*). Since Tyr-tRNA(Phe, yeast) cannot be obtained easily in reasonable yields, we have used p-F-Phe-tRNA(Phe, yeast) as an analogue of the mischarged aa-tRNA. Hydrolysis protection experiments with p-F-Phe-tRNA(Phe, yeast), Phe-tRNA(Lys, *E. coli*), and Lys-tRNA(Lys, *E. coli*) show that p-F-Phe-tRNA(Phe, yeast) is bound more weakly than Phe-tRNA(Phe, yeast), similarily as Tyr-tRNA(Tyr, *E. coli*), and that Phe-tRNA(Lys, *E. coli*) is bound more strongly than Lys-tRNA(Lys, *E. coli*), similarly as for Phe-tRNA(Phe, yeast) (cf. Table I). A similar experiment has been carried out by Sprinzl and Wagner (unpublished experiments). They showed by gel filtration that Phe-tRNA(Lys, *E. coli*) is bound more strongly to EF-Tu·GTP than Lys-tRNA(Lys, *E. coli*). Therefore, we may state that differences in the binding affinity

of different aa-tRNAs toward EF-Tu·GTP are not so much dependent on the nature of the tRNA; they are, however, to considerable extent determined by the amino acid side chain. The tRNA part of the aa-tRNA gives rise to a change of the free enthalpy upon complex formation which is almost the same for all aa-tRNAs. This free enthalpy change is then modulated more or less depending on the nature of the amino acid side chain of the aa-tRNA. The question then is "Which physical property of the amino acid determines whether an aa-tRNA is bound strongly or weakly?" We have therefore determined the binding constants for two other aa-tRNAs to EF-Tu·GTP: Glu-tRNA(Glu, *E. coli*) and Trp-tRNA(Trp, *E. coli*) Table I gives a compilation of the association constants determined for the binding of aa-tRNAs to EF-Tu·GTP by hydrolysis protection experiments under the same conditions. It can be seen that there are two classes of aa-tRNAs, one that binds strongly and the other that binds ~1 order of magnitude more weakly. This division clearly follows a classification of the amino acid into those with polar and those with nonpolar side chains. This result allows us to draw some conclusions as to the nature of the aa-tRNA binding site of EF-Tu·GTP. Part of it must be wide or flexible so as to accommodate amino acid side chains of various sizes. This part is considered to be hydrophobic and, therefore, will favor the binding of aa-tRNAs with unpolar side chains in the aminoacyl group.

It has been suggested by Sprinzl et al. (1977) on the basis of binding experiments with EF-Tu·GTP and Tyr-tRNA(Tyr, *E. coli*), lacking the hydroxyl group in the 2′ or 3′ position, that the major requirement for ternary complex formation is a fixed and identical position of the NH_3^+ group in both isomers. In their model the side chain of the amino acid does not play an important role in the interaction between EF-Tu·GTP and aa-tRNAs. This is in contrast to our experimental finding that the side chain of the aminoacyl group largely determines the differences in affinity of aminoacyl-tRNAs toward EF-Tu·GTP. We have confirmed and quantitated their qualitative result that Tyr-tRNA(Tyr)(3′-dA) binds nearly 10 times more strongly to EF-Tu·GTP than Tyr-tRNA(Tyr)(2′-dA) (M. Sprinzl, A. Pingoud, and C. Urbanke, unpublished experiments) (cf. Table I). This result, however, does not necessarily imply that the side chain is not involved in ternary complex formation. It can easily be demonstrated by model building that one can accommodate both isomers without strain even if the position of the α-carbon and its substituents R–, H–, and NH_3^+– as well as the terminal ribose are in a fixed position (Figure 4a), while the carbonyl group is differently oriented in the two isomers. The different affinity of Tyr-tRNA(Tyr)(3′-dA) and Tyr-tRNA(Tyr)(2′-dA) then may be explained by the fact that the carbonyl group of the ester linkage is involved in ternary complex formation and that the optimum position can only be achieved by the 3′-dA isomer. The more rigid amide bond with its partial double bond character and the lack of extra space precludes the binding of 2′- or 3′-amido analogues of Phe-tRNA(Phe) to EF-Tu·GTP as was established by Sprinzl et al. (1977). This can be due to the fact that the carbonyl moiety has to be rotated out of its optimum position determined from X-ray studies on model compounds (Yathindra & Sundaralingam, 1973) if the location of the amino acid residue is to be similar in both the 2′ and the 3′ isomers. The amide bond cannot perform this rotation because of its partial double bond character.

Contrary to the R substituent of the aminoacyl residue of the aa-tRNA and similar to the NH_3^+ group, the α-hydrogen will be situated in a rather confined part of the binding site

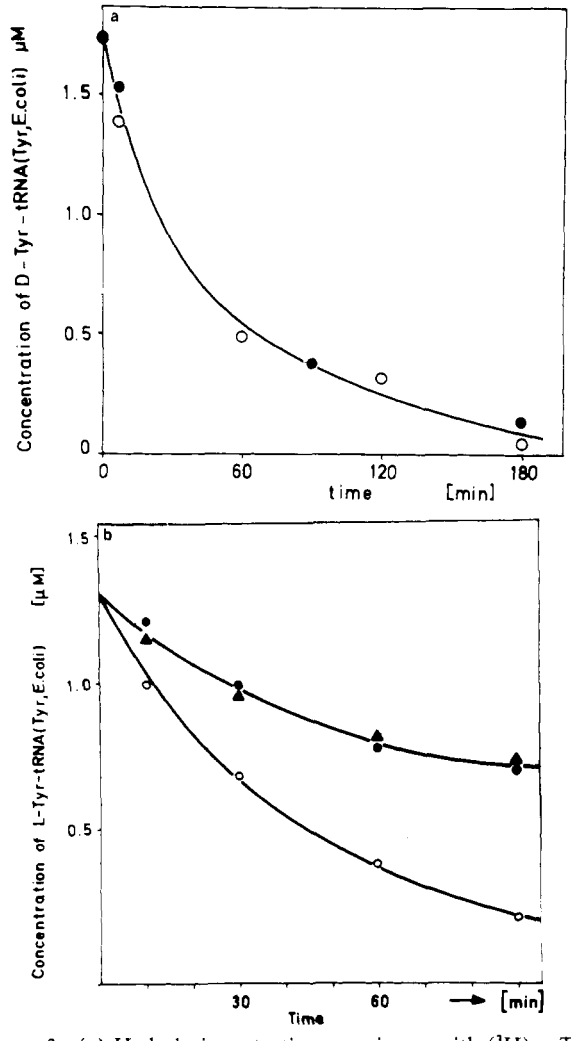

FIGURE 3: (a) Hydrolysis protection experiment with (^{3}H)-D-Tyr-tRNA(Tyr, *E. coli*) at 37 °C. 1.74 µM D-Tyr-tRNA(Tyr, *E. coli*) was incubated without (○) and with (●) 8.77 µM EF-Tu·GTP. (b) Hydrolysis protection experiment with L-Tyr-tRNA(Tyr, *E. coli*), in the presence of D-Tyr-tRNA(Tyr, *E. coli*) at 37 °C 1.3 µM L-(^{14}C)Tyr-tRNA(Tyr, *E. coli*) was incubated without (○) and in the presence of 2 µM EF-Tu·GTP (●, ▲). (●) denotes the control experiment without D-Tyr-tRNA(Tyr, *E. coli*); (▲) is the competition experiment with additional 17 µM D-Tyr-tRNA(Tyr, *E. coli*).

which will not accommodate large substituents. This can be derived from hydrolysis protection experiments with D-Tyr-tRNA(Tyr, *E. coli*). Figure 3a shows that D-Tyr-tRNA(Tyr, *E. coli*) is not protected by EF-Tu·GTP against hydrolysis. Furthermore, D-Tyr-tRNA(Tyr, *E. coli*) in more than 10-fold excess does not compete with L-Tyr-tRNA(Tyr, *E. coli*) for its binding site on EF-Tu·GTP (Figure 3b). Therefore, we conclude that the binding of D-Tyr-tRNA(Tyr, *E. coli*) to EF-Tu·GTP can only have an equilibrium constant of $<10^5$ M^{-1}. It is this selectivity of EF-Tu·GTP that prevents tRNAs that are misacylated with D-amino acids to be transferred to the ribosome. Furthermore, if there is no other selection taking place on the ribosome, EF-Tu·GTP prevents due to the stereochemistry of its binding site the incorporation of D-amino acids into proteins or at least blocking of the ribosomal protein biosynthesis by D-aminoacyl-tRNAs. The finding of Calendar & Berg (1967) that D-tyrosine is incorporated into protein using a cell-free extract of *Bacillus subtilis* and excess D-Tyr-tRNA(Tyr, *E. coli*) can be explained by a low binding constant or by consideration of factor-free binding of D-Tyr-tRNA(Tyr, *E. coli*) to the ribosome. Correspondingly, under conditions where L-Tyr-tRNA(Tyr) is present, there will be

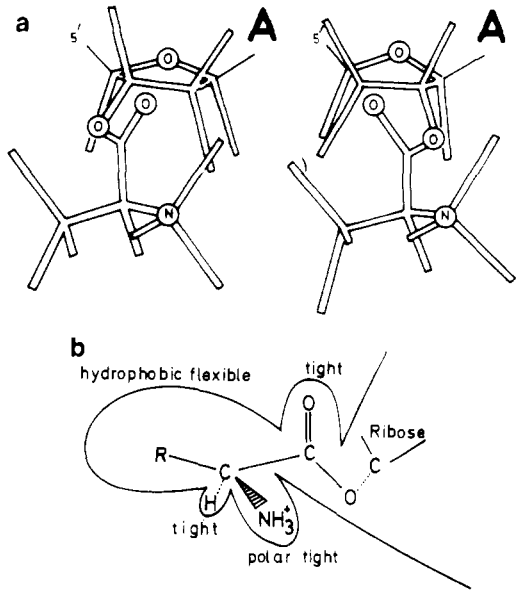

FIGURE 4: In (a), a perspective drawing of a skeletal model of the aminoacylated 3′ terminus of an aminoacyl-tRNA(dA) is seen. For clarity only the aminoacylated deoxyribose ring is shown. The L-alanyl residue is shown esterified to the 2′ position with the NH_3-group in front, the CH_3-group oriented slightly backward, and the -H oriented downward. The position of these substituents relative to the ribose ring is almost identical for both isomers. The binding site of EF-Tu is considered to be complementary to this conformation, providing close contacts around the NH_3-group, the ester linkage, and the α-hydrogen and a flexible groove or pocket for the amino acid side chain. The part of the aminoacyl-tRNA binding site of the EF-Tu which accommodates the aminoacyl moiety is depicted schematically in (b).

no incorporation of D-tyrosine into proteins.

A summarized view of the nature of the aminoacyl-tRNA binding site is given in the diagrammatic illustration in Figure 4b. It should be emphasized, however, that our conclusion that the aminoacyl residue is important for the differences in ternary complex formation is based on investigations of only a limited number of tRNA species. Experiments with other tRNAs will help to refine the model.

Outlook

We have established that naturally occurring aminoacyl-tRNAs have different affinities toward the elongation factor Tu from *E. coli*. It was pointed out by us recently that this does not lead to a preferential supply of the strongly binding aminoacyl-tRNAs to the ribosome since the intracellular amount of the elongation factor Tu is sufficient to complex all of the aminoacyl-tRNAs present in an *E. coli* cell (Pingoud et al., 1977). Consequently, the observed differences in ternary complex formation are most likely to be of no consequence for in vivo protein biosynthesis. They have allowed us, however, to define morphological features of a part of the aminoacyl-tRNA binding site of the elongation factor Tu.

We still cannot explain why uncharged tRNAs are bound so weakly or why aminoacylated tRNA fragments are bound by at least 3 orders of magnitude more weakly than the natural substrate. A clue for the solution of this problem is provided by the experiments of Schulman et al. (1974) and Jekowsky et al. (1977), which indicate that the 5′-terminal phosphate and a base-paired acceptor stem are needed for normal ternary complex formation. We will extend our present studies on the aminoacyl-tRNA binding site of the elongation factor Tu by an investigation of the quantitative aspects of the binding of aminoacylated tRNAs and tRNA fragments modified in the acceptor stem region, which will possibly allow us to define the contributions of other structural elements of aminoacyl-tRNAs than the aminoacyl residue to the overall free enthalpy change upon ternary complex formation. It might, however, be necessary to consider conformational differences between charged and uncharged tRNAs as well as/or conformational changes of the ternary complex triggered by a specific structural property of the aminoacyl-tRNA.

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