Some Molecular Properties of the Elongation Factor EF1 from Wheat Embryos†

R. Bollini, A. N. Soffientini, A. Bertani, and G. A. Lanzani*†

ABSTRACT: Elongation factor EF1 from wheat embryos exists in two forms of different molecular weights ($EF1_H$ and $EF1_L$). We now report that the molecular weights of $EF1_H$ and $EF1_L$ determined by analytical ultracentrifugation are 187,000 ± 5000 and 51,000 ± 2000, respectively. Sodium dodecyl sulfate disc electrophoresis indicates that in $EF1_H$ three peptides A, B, and C are present. The molecular weights of A, B, and C are respectively 52,000, 47,000, and 27,000. In $EF1_L$ only peptide A is present.

It is known that the elongation factor EF1 is present in wheat embryos in two forms of different molecular sizes: a high molecular weight species ($EF1_H$) and another having lighter molecular weight ($EF1_L$) (Tarrago' *et al.*, 1973, Golinska and Legocki, 1973). These two different forms of wheat embryo EF1 have different enzymatic properties; while $EF1_H$ cannot directly form the ternary complex aminoacyl-tRNA-GTP-EF1, both $EF1_H$ and $EF1_L$ can catalyze the binding of Phe-tRNA to ribosomes in the presence of poly(U) (Legocki *et al.*, 1974). The results till now obtained suggest that $EF1_H$ may represent either a mere storage form of $EF1_L$ or perhaps a regulatory form. We found that incubating $EF1_H$ with Phe-tRNA and GTP in the conditions for the formation of the ternary complex, a portion of $EF1_H$ is transformed in $EF1_L$, and only $EF1_L$ is comprised in the ternary complex (Lanzani *et al.*, 1974a). The disaggregation $EF1_H \rightarrow EF1_L$ is influenced by cGMP, and during this transformation a form of EF1 of intermediate molecular size is formed (Lanzani *et al.*, 1974b). The mechanisms of the transformation $EF1_H \rightarrow EF1_L$ are difficult to understand until something more about the molecular characteristics of $EF1_H$ and $EF1_L$ will be known.

This paper is concerned with the determination by analytical ultracentrifugation of the molecular weights of $EF1_H$ and $EF1_L$; the peptides contained in the two types of EF1 were determined by SDS[1] polyacrylamide electrophoresis. Based on these data some considerations about the molecular relations between the different forms of EF1 will be discussed.

Materials and Methods

Preparation of $EF1_H$ and $EF1_L$. A quantity of $EF1_H$ corresponding to 15 mg of $EF1_H$, prepared as already described (Lanzani *et al.*, 1974a), was passed through a second hydroxylapatite column (20 × 1 cm) conditioned with 50 mM potassium phosphate and 1 mM dithiothreitol buffer (pH 7.5) (buffer A), and eluted with a linear gradient formed by 100 ml of buffer A and 100 ml of 300 mM potassium phosphate and 1 mM dithiothreitol buffer (pH 7.5). A flow rate of 10 ml/hr was maintained and fractions of 0.5 ml were collected at 0°. The fractions were tested for the GTP binding activity and the active fractions were pooled.

$EF1_L$ was prepared by incubating $EF1_H$ in the conditions for the formation of the ternary complex; 10^{-3} M [^{3}H]GTP, 11,000 pmol of [^{14}C]Phe-tRNA, and 8000 units of $EF1_H$ (determined with the method of Allende and Ofengand, 1971) were used in 1 ml. After incubation for 10 min at 32° and 10 min at 0° the reaction mixture was gel filtrated on a Sephadex G-200 column (24 × 2 cm) conditioned with 50 mM Tris-HCl, 10 mM $MgCl_2$, 60 mM NH_4Cl, and 1 mM dithiothreitol buffer (pH 7.5), and eluted with the same buffer; with a flow rate of 10 ml/hr, fractions of 1 ml were collected. The peak corresponding to the GTP binding activity eluted with an elution volume similar to bovine serum albumin and contained the ternary complex and free [^{14}C]Phe-tRNA as already described (Lanzani *et al.*, 1974a); it was collected, precipitated at 80% $(NH_4)_2SO_4$ saturation, dissolved in 50 mM sodium acetate, 200 mM NaCl, 10 mM $MgCl_2$ buffer (pH 5.5) (buffer B), and dialyzed against buffer B. This solution was put on a benzoylated DEAE-cellulose column (7 × 1 cm) conditioned with buffer B in order to dissociate the ternary complex (Klyde and Bernfield, 1973). The elution was performed with buffer B at a flow rate of 10 ml/hr; 0.5-ml fractions were collected at 0°. The fractions containing $EF1_L$ were pooled, dialyzed against 150 mM potassium phosphate and 1 mM dithiothreitol buffer (pH 7.5) (buffer C), and concentrated by dialysis against dry Sephadex G-200 powder.

Ultracentrifugation. Sedimentation velocity and equilibrium studies were carried out in a Beckman Model E analytical ultracentrifuge. Schlieren optics were used for sedimentation velocity determinations; for sedimentation, equilibrium recorder traces were obtained with the photoelectric scanning absorption system at 280 nm.

The partial specific volume of $EF1_H$ was determined with the equilibrium centrifugation method either in water (buffer C) or in D_2O solution (Edelstein and Schachman, 1967). The D_2O solution contained 90% D_2O and 10% buffer C. In these experiments two double-sector cells were used with multiplex operation of the photoelectric scanner. About 0.01 ml of fluorocarbon FC43 was added to the compartment of the double-sector cell containing 0.09 ml of the sample, in order to produce a transparent region at the cell bottom and thereby permit accurate measurement of the absorbance throughout the liquid column.

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[1] Abbreviation used is: SDS, sodium dodecyl sulfate.

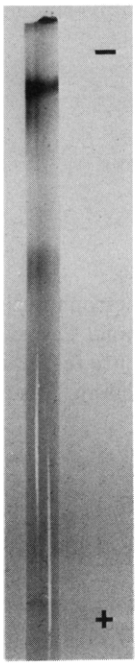

FIGURE 1: Disc gel electrophoresis of $EF1_H$ preparation. Gels were run at 2 mA/tube at 13° until Bromophenol Blue ran from the top to the bottom of the gel. Gels were stained with Coomassie Blue in 1% trichloroacetic acid solution and destained with 10% trichloroacetic acid; 150 μg of protein was used.

Polyacrylamide Disc Gel Electrophoresis. Purity of the $EF1_H$ preparation was tested by electrophoresis on 7% acrylamide gels, at pH 8.3, at 13°, as described by Davis (1964).

The peptide composition of $EF1_H$ and $EF1_L$ was determined by electrophoresis in the presence of 0.1% sodium dodecyl sulfate and 4 mM urea, according to the procedure of Weber and Osborn (1969). Marker peptides were catalase, aldolase, and cytochrome *c;* the molecular weights of their polypeptide chains being 60,000, 40,000, and 11,700, respectively.

[^{14}C]Phe-tRNA Preparation. [^{14}C]Phe-tRNA was prepared from partially purified $tRNA^{Phe}$ of wheat embryos by the method of Vold and Sypherd (1968). The product contained 176 pmol of [^{14}C]Phe-tRNA/A_{260nm} unit.

EF1 Activity. The EF1 activity was assayed measuring either the binding of [^{3}H]GTP to EF1 according to Allende and Ofengand (1971), or the binding of [^{14}C]Phe-tRNA to ribosomes (method of Nirenberg and Leder, 1964).

Preparation of Ribosomes. Crude ribosomes, prepared as already described (Lanzani *et al.,* 1974a), were purified from contaminating factors by chromatography on a DEAE-cellulose column (Lanzani and Soffientini, 1973).

Protein Concentration. The protein concentration was determined according to the method of Warburg and Christian (1941). For some experiments, protein concentration was also determined by the method of Lowry *et al.* (1951), using crystalline bovine serum albumin as a standard.

[^{14}C]Phenylalanine and [^{3}H]GTP were purchased from NEN; GTP, catalase, aldolase, cytochrome *c,* and bovine serum albumin, from Boehringer; acrylamide, *N,N,N,N'*-tetramethylethylenediamine and ammonium persulfate, from Bio-Rad; Sephadex G-200 and G-25, from Pharmacia. The nitrocellulose filters were Millipore H.A.W.P., 0.45 mμ pore diameter. The other chemicals were analytical grade from Merck.

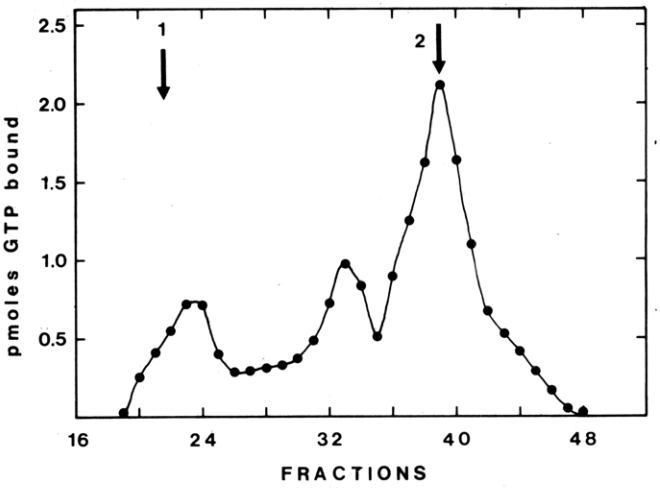

FIGURE 2: Sephadex G-200 gel filtration after incubation of $EF1_H$ with GTP and Phe-tRNA. The column was charged with 1 ml of complex, formed as described in Materials and Methods; 0.050-ml aliquots of each fraction were used for EF1 activity determination (●). The elution volume of blue dextran (arrow 1), and bovine serum albumin (arrow 2). The elution peak of free GTP was at fraction 61.

TABLE I: Enzymatic Characteristics of $EF1_H$ and $EF1_L$.

	$EF1_H$	$EF1_L$
Phe-tRNA picomoles bound to ribosomes (I)	160	50
GTP picomoles bound to EF1 complex (II)	11	38
Ratio I/II	14.6	1.3

Results

Preparation of $EF1_H$ and $EF1_L$. $EF1_H$ was prepared as already described by ammonium sulfate fractionate precipitation, Sephadex G-200 gel-filtration, and hydroxylapatite chromatography (Lanzani *et al.,* 1974a); after a second hydroxylapatite chromatography, the $EF1_H$ preparation gave on polyacrylamide gel electrophoresis a single band representing more than 95% of the protein used and a very slight band indicating less than 5% of impurities, as shown in Figure 1.

$EF1_L$ was prepared incubating $EF1_H$ in the conditions for the formation of the ternary complex Phe-tRNA-GTP-$EF1_L$; the ternary complex was isolated by gel filtration on a Sephadex G-200 column (Figure 2). We used Sephadex G-200 instead of Sephadex G-150, because the Sephadex G-200 allowed a better separation of the complex Phe-tRNA-GTP-$EF1_L$ from the remaining different forms of disaggregation of $EF1_H$. The fractions 37–41 containing the Phe-tRNA-GTP-$EF1_L$ complex together with free Phe-tRNA were collected, put on a benzoylated DEAE-cellulose column, and eluted as described in the Materials and Methods section. The ternary complex lost Phe-tRNA and GTP, which were retained on the column (Klyde and Bernfield, 1973), while $EF1_L$ was eluted; the fractions containing $EF1_L$ were collected, dialyzed against buffer C, and concentrated by dialysis against dry Sephadex G-200 powder. Some functional properties of $EF1_H$ and $EF1_L$ are described in Table I. These data show that the ratio picomoles of Phe-tRNA bound to ribosomes/picomoles of GTP bound in the complex GTP-EF1 is 14.6 for $EF1_H$ and

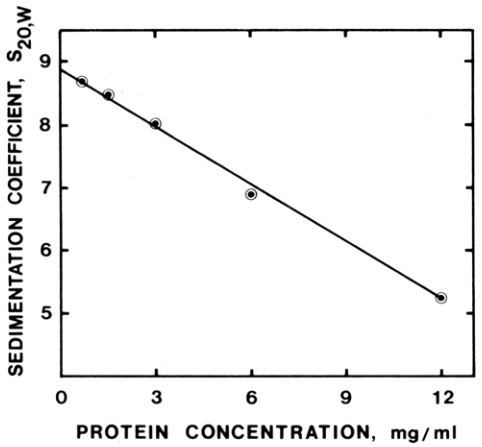

FIGURE 3: Sedimentation coefficient of the binding enzyme as a function of protein concentration. $s_{20,w}$ values were obtained at concentrations of 0.69–12.00 mg/ml of protein in buffer C (see Materials and Methods section), and 52,000 rpm. Calculations were corrected to distilled water at 20°.

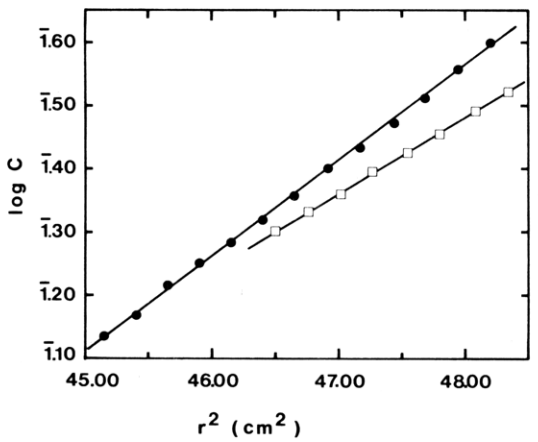

FIGURE 4: Sedimentation equilibrium of $EF1_H$. In the experiments here shown the concentration of water solution was $0.450A_{280nm}$, that of D_2O solution was $0.400A_{280nm}$. The speed was 6,000 rpm for 24 hr and the temperature was 5.3°. Measurements were made from expanded traces obtained with the photoelectric scanning absorption system, and the data were plotted as the A_{280nm} logarithm against the square of the distance in centimeters (r) from the axis of rotation. A molecular weight of 187,353 was obtained. (●) water solution; (□) D_2O solution.

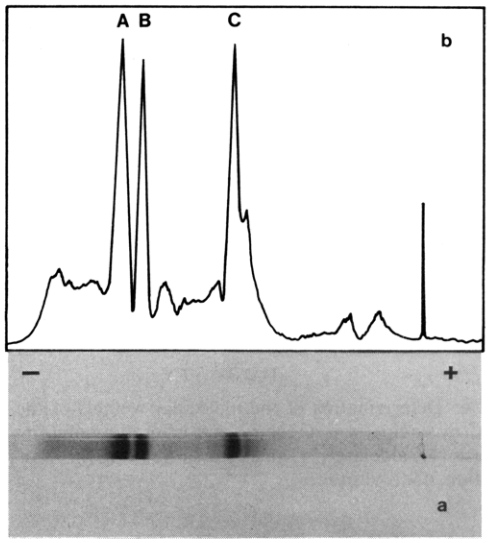

FIGURE 5: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of $EF1_H$ preparation; 100 μg of $EF1_H$ was incubated with 4 M urea, 1% sodium dodecyl sulfate, and 1% 2-mercaptoethanol for 2 hr at 37°, applied to the 10% gel (9.5 × 0.5 cm), and electrophorezed in the presence of 0.1% sodium dodecyl sulfate. (a) The photo of gel; (b) the scanning at 568 nm.

TABLE II: Sedimentation Coefficient Determination.

Run	Rotor	Temp (°C)	EF1 (mg/ml)	$s_{20,w}$
1	AN-D	8.75	12.00	5.25
2	AN-D	6.85	6.00	6.82
			3.00	8.02
3	AN-E	6.95	1.50	8.50
			0.69	8.70

about 1 for $EF1_L$. This fact might indicate either that $EF1_H$ can recycle after the binding of AA-tRNA to ribosomes, whereas $EF1_L$ cannot, or that the function of binding AA-tRNA to ribosomes in $EF1_L$ is very labile.

Sedimentation Behavior of $EF1_H$ and Molecular Weights of $EF1_H$ and $EF1_L$. The purified preparation of $EF1_H$ sediments as a single protein boundary (Lanzani *et al.*, 1974a). The sedimentation rate varies with the protein concentration as indicated by the plot of sedimentation coefficient against protein concentration presented in Figure 3. Extrapolation of the plot to zero protein concentration indicates that the $s_{20,w}$ for $EF1_H$ is 8.9. In Table II the experimental conditions of these determinations are reported.

The molecular weights of $EF1_H$ and $EF1_L$ were determined by sedimentation equilibrium with the method of Edelstein and Schachman (1967). Figure 4 represents the sedimentation equilibrium of $EF1_H$ either in water or D_2O. No deviation from linearity of the curve log (C/r^2) is observed. A partial specific volume of 0.777 ± 0.002 was found for $EF1_H$ using the formula of Edelstein and Schachman (1967). Using this value, a molecular weight of 187,000 ± 5000 can be assigned to $EF1_H$. For the determination of molecular weight of $EF1_L$, the solution contained 0.400 A_{280nm} unit in buffer C. Speed of 10,000 rpm and a temperature of 16.8° were used; the centrifugation was performed during 24 hr; measurements were made as described in the legend of Figure 4. Also for $EF1_L$ there was no deviation from linearity of the curve log (C/r^2). A value of 51,000 ± 2000 was obtained for $EF1_L$ using the same partial specific volume as $EF1_H$.

Peptide Composition of $EF1_H$ and $EF1_L$. The SDS gel electrophoresis of $EF1_H$, using the method of Weber and Osborn (1969), evidenced the presence of three different peptides: A, B, and C (Figure 5a); the areas of the corresponding peaks obtained by scanning the electrophoresis tube at 568 nm are quantitatively similar (Figure 5b). The fact that the amounts of peptides A, B, and C found in $EF1_H$ were very similar, as indicated by the scanning patterns, excludes that B and C are derived from the impurities present in the preparation of $EF1_H$.

The molecular weights of the three peptides, determined by comparison of their mobilities with those of marker peptides, are 52,000, 47,000, and 27,000 for A, B, and C, respectively, as shown in Figure 6. In Figure 7 the SDS electrophoretical patterns of $EF1_H$ and $EF1_L$ are compared. Of

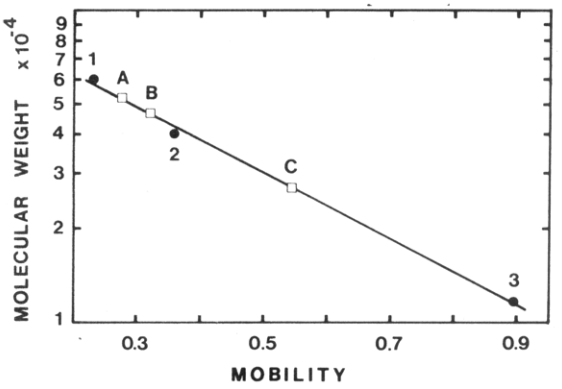

FIGURE 6: Determination of the molecular weights of the peptide A, B, and C by comparison of their electrophoretic mobilities with those of catalase (1), aldolase (2), and cytochrome *c* (3), in the presence of 0.1% sodium dodecyl sulfate.

the three bands present in $EF1_H$ only the one corresponding to peptide A is present in $EF1_L$; the data of SDS electrophoresis already described indicate for peptide A a molecular weight of 52,000. This value agrees fairly well with the molecular weight of $EF1_L$ determined by analytical ultracentrifugation of 51,000. Besides the band corresponding to peptide A, another band corresponding to a dimer of A is present in $EF1_L$ electrophoretic pattern. This dimer of A was probably formed during the SDS treatment of $EF1_L$ because it was not observed during the sedimentation equilibrium determination (no deviation of linearity of the curve log (C/r^2)).

Discussion

The analytical ultracentrifugation data indicate that $EF1_H$ from wheat embryos has a molecular weight of 187,000 ± 5000. This value was rather unexpected, because $EF1_H$ is eluted in the void volume of a Sephadex G-200 column, distinctly before the catalase (240,000) (Lanzani *et al.*, 1974a). This anomalous behavior during Sephadex G-200 gel filtration might reflect some particular chemical or physical characteristic of $EF1_H$. By SDS electrophoresis we found in $EF1_H$ three different peptides, A, B, and C, whose molecular weights were 52,000, 47,000, and 27,000, respectively. From $EF1_L$ only peptide A was obtained. These data are at variance with those of Golinska and Legocki (1973), who found only one peptide in $EF1_H$. Probably during their EF1 preparation peptides B and C have been separated from A. Functions of peptides B and C have not yet been established; at least they are necessary for the stabilization of the peptide A, which in their absence is extremely unstable and dimerizes and trimerizes. That A, B, and C are copurified as a single aggregate might be merely fortuitous, but the fact that many procedures of purification fail to resolve this aggregation and only an act of the functioning of EF1 (the formation of the ternary complex) results in the detaching of A from B and C could imply particular relations between A, B, and C.

The hypothesis that $EF1_H$ represents a regulatory form is corroborated by the data indicating that cGMP activates the poly(Phe) synthesis in the wheat embryos *in vitro* system by influencing the $EF1_H$ disaggregation (Lanzani *et al.*, 1974b). The transformation of $EF1_H \rightarrow EF1_L$ during the formation of the ternary complex consists in the separation of two peptides (B and C) from $EF1_H$ and the single peptide A enters the complex. The data till now obtained on the molecular properties of wheat embryo $EF1_H$ and $EF1_L$,

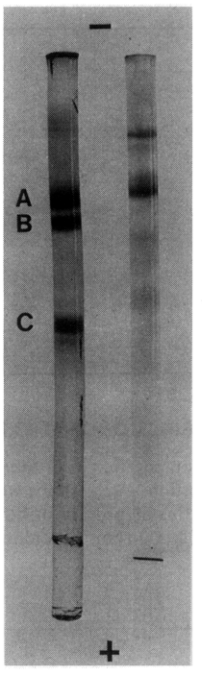

FIGURE 7: Comparison of sodium dodecyl sulfate gel electrophoresis of $EF1_H$ and $EF1_L$. For $EF1_L$ 30 μg was used and the electrophoresis was performed as described for $EF1_H$ (see Figure 5).

and the fact that $EF1_H$ passes through a form of lighter molecular weight during the formation of the ternary complex, loosing two of its three peptides, agree to indicate that wheat embryo EF1 undergoes a cycle during its functioning. EF1 from calf brain undergoes a similar cycle $EF1_H \rightleftarrows EF1_L$ (Moon *et al.*, 1972); however, in this case the cycle seems constituted by a simple process of aggregation, disaggregation of a single peptide. The presence of different peptides in $EF1_H$ may be in line with what has been found for another eukaryote EF1 by Prather *et al.* (1974), who demonstrated that EF1 from reticulocytes is composed of two components, one having the functions of EF-Tu and another of EF-Ts of *Escherichia coli*. The idea that the recycling of EF1 after the binding of aminoacyl-tRNA to the ribosomes needs B and C is supported by the data reported in Table I: $EF1_L$ in the absence of B and C is able to operate only one binding of aminoacyl-tRNA to ribosomes and cannot recycle (ratio AA-tRNA bound to ribosomes/GTP bound to $EF1_L$ near 1), whereas $EF1_H$ recycles (AA-tRNA bound to ribosomes/GTP bound to $EF1_H$ = 14.6). The definitive proof that the recycling of $EF1_L$ needs B and C would be the reconstitution of $EF1_H$ from $EF1_L$, B, and C, but as yet B and C have been isolated as SDS denatured and not as native peptides.

Moreover data not reported indicate that ribosomes are involved in the recycling of $EF1_L$ to $EF1_H$; the mechanism of the process appears rather complex and further studies are needed for a complete clarification.

Acknowledgments

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Enzymatic Acylation of Oxidized–Reduced Transfer Ribonucleic Acid by *Escherichia coli,* Yeast, and Rat Liver Synthetases Occurs Almost Exclusively at the 2′-Hydroxyl†

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ABSTRACT: The position of acylation in seven tRNA synthetase systems was examined. Enzymatic acylation with phenylalanine of the nonisomerizable tRNA analog, oxidized–reduced tRNA, made it possible to isolate a stable 3′-terminal fragment. Reaction with *N*-benzyloxycarbonyl chloride, followed by RNase hydrolysis, yielded *N*-benzyloxycarbonylphenylalanyladenosine$^{ox\text{-}red}$ which was separated into its 2′ and 3′ isomers by thin-layer chromatography. When yeast synthetase attached phenylalanine to yeast tRNA^{Phe} under normal conditions, or to *E. coli* tRNA^{Phe} or *E. coli* tRNA^{Val} in the presence of 20% dimethyl sulfoxide, 98–99% of the product was the 2′ isomer. When *Escherichia coli* synthetase acylated *E. coli* tRNA^{Phe} normally, or yeast tRNA^{Phe} in the presence of 1.5 M ammonium sulfate, 95–97% of the product was the 2′ isomer. Acylation of rat liver tRNA^{Phe} or yeast tRNA^{Phe} by rat liver synthetase gave 90–92% 2′ isomer. The results with the yeast synthetase–yeast tRNA^{Phe} system were verified by nuclear magnetic resonance analysis which showed that >85% of the product was the 2′ isomer. Retention of a high degree of specificity for the 2′-hydroxyl under such widely varying conditions leads to the conclusion that this is the normal site of acylation in the biosynthesis of aminoacyl tRNA.

In a previous publication from this laboratory, the activity of Phe-tRNA$^{ox\text{-}red}$ [1] prepared by enzymatic acylation of oxidized–reduced yeast tRNA^{Phe} was examined in several intermediate reactions of protein synthesis (Ofengand and Chen, 1972). This modification was found to block EFTu-GTP recognition, binding to the A site on the ribosomes, and to diminish its affinity for nonenzymatic binding at the ribosomal P site. At that time, we suggested the possibility that the reason for this lack of activity lay not in the cleavage of the $C_{2'}$–$C_{3'}$ bond but was due to enzymatic acylation of the 2′-hydroxyl, failure to isomerize due to the lack of vicinal hydroxyl groups in the opened ribose ring, and a specificity for 3′-esterified AA-tRNA in protein synthesis.

Subsequent work (Hussain and Ofengand, 1973) confirmed this hypothesis by the demonstration that chemically synthesized Ado$^{ox\text{-}red}$(Phe) [IV, Figure 1], a mixture of the 2′ and 3′ isomers, was active as a peptidyl transferase acceptor while biologically synthesized Ado$^{ox\text{-}red}$(Phe) and CACCA$^{ox\text{-}red}$(Phe), prepared from enzymatically acylated tRNA$^{ox\text{-}red}$, were completely inactive. Since puromycin, a 3′-analog of aminoacyl-tRNA, is the classic acceptor for peptidyl transferase, it was concluded (a) that peptidyl transferase was 3′ specific, (b) that enzymatic acylation was 2′ specific, and (c) that isomerization between 3′ and 2′ does not occur in the open ring ester system.

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[1] Abbreviations used are: tRNA$^{ox\text{-}red}$, tRNA which has been first oxidized at the 3′-terminal end with periodate, and then reduced to the diol by $NaBH_4$; Ado$^{ox\text{-}red}$, adenosine modified as above, the correct nomenclature is 2-(adenin-9-yl)-4′-methylol-3-oxopentane-1,5-diol (see Figure 1, $R_1 = R_2 = R_3 = H$); EFTu, one of the peptide chain elongation factors from *E. coli;* Z, *N*-benzyloxycarbonyl; MeOTr, *p*-methoxytrityl; A_{260}, that amount of tRNA in 1 ml which has an absorbance at 260 nm of 1.0 in a 1-cm path cell; Ado(Z-Phe), a mixture of Ado(2′-Z-Phe) and Ado(3′-Z-Phe); Ado(Phe), a mixture of Ado(2′-Phe) and Ado(3′-Phe).