Effect of *Escherichia coli* Initiation Factors on the Kinetics of *N*-AcPhe-tRNA^{Phe} Binding to 30S Ribosomal Subunits. A Fluorescence Stopped-Flow Study†

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ABSTRACT: The mechanism of binding of *N*-AcPhe-tRNA^{Phe} (yeast) to poly(U)-programmed *Escherichia coli* 30S ribosomal subunits and the effect of individual initiation factors (IF-1, IF-2, and IF-3) and GTP on this process have been studied by fluorescence stopped-flow kinetic measurements. The formation of the ternary complex was followed by an increase of both intensity and polarization of the fluorescence of a proflavin label located in the anticodon loop of the tRNA. The effect of the initiation factors and GTP is to increase the velocity of ternary complex formation (about 400-fold at 7 mM Mg^{2+}). In the presence of the three initiation factors and GTP the formation of the ternary complex could be resolved into two partial reactions: a fast apparently second-order step (k_{12} = 5 × 10^6 M^{-1} s^{-1}, k_{21} = 1.4 s^{-1}) followed by a slow rearrangement step ($k_{23} \leq 0.1$ s^{-1}). The data suggest a mechanism in which the ternary complex is formed by at least two rearrangements of an initially formed preternary complex. The accelerating effects of both IF-2 and IF-3 can be understood by assuming a synergistic allosteric action of the factors on the 30S ribosomal subunit, whereas IF-1 appears to act indirectly by influencing the other two factors.

During initiation of protein biosynthesis in prokaryotes a ternary complex consisting of 30S ribosomal subunits, mRNA, and initiator tRNA is formed. Three protein factors (IF-1, IF-2, and IF-3)[1] as well as one molecule of GTP participate in the formation of the complex. However, the roles played by these molecules and several mechanistic aspects of the process still remain obscure (Grunberg-Manago, 1980; Gualerzi & Pon, 1981). Initial rate kinetic measurements carried out by using the nitrocellulose filtration assay showed that there is no obligatory order of substrate binding (mRNA or aminoacyl-tRNA) to 30S ribosomal subunits. Thus a random binding mechanism was proposed in which two binary complexes (30S–mRNA and 30S–aminoacyl-tRNA) and a preternary complex are in rapid equilibrium and precede the rate-determining step which transforms the preternary complex into the stable ternary complex (Gualerzi et al., 1977).

In order to study more directly and more precisely the step(s) preceding the rate-limiting step and to obtain information concerning the role played by the individual initiation factors, we have carried out stopped-flow kinetic experiments. The formation of the 30S initiation complex has been studied by using a model system in which the binding of *N*-AcPhe-tRNA^{Phe} to poly(U)-programmed 30S ribosomal subunits could be monitored by a fluorescence change of a proflavin covalently bound next to the anticodon (Wintermeyer & Zachau, 1979). The choice of such a model system for our studies is justified by the well-documented similarity between this ternary complex and the physiological 30S initiation complex as far as factor requirements and mechanistic aspects are concerned (Lucas-Lenard & Lipmann, 1967; Bernal et al., 1974; Gualerzi et al., 1977; Blumberg et al., 1979).

Materials and Methods

Biochemicals. Ribosomal subunits (30S) have been prepared from *Escherichia coli* MRE 600 tight couple ribosomes (Noll et al., 1973) by zonal centrifugation and washed as described previously (Risuleo et al., 1976). According to the protein analysis by $NaDodSO_4$ electrophoresis (Laemmli, 1970; Studier, 1973) and densitometric quantitation of the stained bands using protein S4 as an internal standard and assuming that unwashed 30S ribosomal subunits contain one copy of S1, the 30S particles used for the present experiments contained on the average 0.7 copy of S1. For some particular experiments (see Results) the amount of S1 was reduced by successive salt-washing steps. By the above criteria, these 30S ribosomes contained 0.3 copy of S1. About 50% of the 30S ribosomal subunits were active in poly(U)-dependent binding of *N*-AcPhe-tRNA. The three *E. coli* initiation factors (Pawlik et al., 1981) and *N*-AcPhe-tRNA$^{Phe}_{Prf37}$ from yeast[1] (1.5 nmol of *N*-AcPhe/A_{260} unit) (Robertson & Wintermeyer, 1981) have been prepared as described in the references.

The concentrations of ribosomes and tRNA have been determined from absorption measurements at 260 nm by using extinction coefficients (μM^{-1} cm^{-1}) of 14.3 and 0.595, respectively. The concentrations of initiation factors were determined by the folin reaction (Lowry et al., 1951) with lysozyme as standard.

Stopped-Flow Experiments. Unless indicated otherwise, the experiments have been performed at 20 °C in a buffer containing 20 mM Tris-HCl, pH 7.5, 100 mM ammonium chloride, 3 mM 2-mercaptoethanol, and 5–15 mM magnesium acetate, as indicated; 30S ribosomal subunits (heat activated at 50 °C for 5 min) were incubated with poly(U) (final concentration after mixing with tRNA 2.4 A_{260} units/mL). GTP (final concentration 0.5 mM) and the initiation factors (concentrations equimolar to the 30S ribosomal subunits) were incubated for 5 min at 20 °C before the experiment. The solution of *N*-AcPhe-tRNA$^{Phe}_{Prf37}$ after dilution with the same buffer was allowed to stand at 4 °C for 30 min before the experiment; deacylation was negligible under these conditions.

Complex formation was initiated by rapidly mixing (mixing time less than 5 ms) equal volumes (about 80 μL each) of the

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[1] Abbreviations: *N*-AcPhe-tRNA$^{Phe}_{Prf37}$, *N*-acetylphenylalanyl-tRNA^{Phe} (yeast) in which wybutine at position 37 next to the anticodon is replaced with proflavin; IF, initiation factor; Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate.

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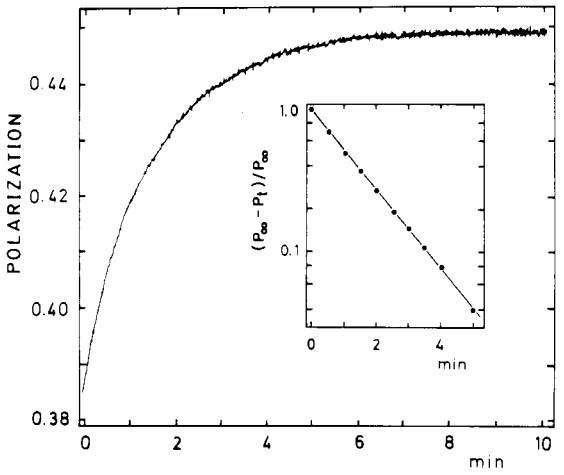

FIGURE 1: Ternary complex formation of *N*-AcPhe-tRNA$^{Phe}_{Prf37}$ with poly(U)-programmed 30S ribosomal subunits without initiation factors. The stopped-flow experiment was performed at 15 mM Mg^{2+} with *N*-AcPhe-tRNA$^{Phe}_{Prf37}$ (2×10^{-8} M final concentration) and 30S subunits (1.8×10^{-7} M) which had been preincubated with poly(U) as described under Materials and Methods. Because the fluorescence of proflavin is not stable enough to allow very long measurements, only the polarization of fluorescence, which is independent of the concentration of the fluorophor, has been measured. The inset shows the semilogarithmic plot of the data which yields a single exponential, characterized by a relaxation time of around 100 s.

solutions containing the tRNA and the ribosomes, respectively. The stopped-flow apparatus has been described in detail elsewhere (Wintermeyer & Robertson, 1982). Proflavin fluorescence was excited at 436 nm and measured with two photomultiplier tubes after passing cutoff filters (Schott KV 500). For polarization measurements, polarizing foils were inserted into the excitation and the two emission light paths.

Data Evaluation. All kinetic experiments have been performed at an at least 5-fold excess of 30S subunits (and initiation factors) over the tRNA, i.e., under pseudo-first-order conditions. Therefore, the reaction progress curves, as measured by the changes of either intensity or polarization of fluorescence, represent exponential decay curves. Relaxation times and amplitudes have been obtained by single- or two-exponential least-squares parameter fitting as previously described (Wintermeyer & Robertson, 1982).

Results

Effect of IF-3 on Ternary Complex Formation. In order to have a comparison to previous data obtained by the nitrocellulose filtration assay (Gualerzi et al., 1977), we first studied the effect of IF-3 on the rate of binding of *N*-AcPhe-tRNA$^{Phe}_{Prf37}$ to poly(U)-programmed 30S ribosomal subunits. In the fluorescence stopped-flow experiments, the formation of the ternary complex could be followed by an increase of both intensity and polarization of the proflavin fluorescence.

In the absence of any initiation factor the complex forms rather slowly, the relaxation time (see Materials and Methods) being about 100 s at 15 mM Mg^{2+} (Figure 1). At lower Mg^{2+} concentrations the rate of complex formation is even lower, the relaxation time being around 6 min at 7 mM Mg^{2+} (not shown). This is comparable to the rate of complex formation measured by the filtration assay in the absence of factors (Gualerzi et al., 1977).

The addition of IF-3 considerably enhances the rate of ternary complex formation (Figure 2), about 20-fold under the present conditions. (As will be discussed below, there is a strong influence of Mg^{2+} on the rate of complex formation.) The reaction progress curves can be described by one exponential function (cf. fitted curves in Figure 2), suggesting that a single reaction is being monitored by both observables. Due to the IF-3-induced destabilization of this complex (Gualerzi et al., 1979) the amount of complex and, with that, the amplitude of the signal change are smaller in the presence than in the absence of the factor (compare the final polarization values in Figures 1 and 2).

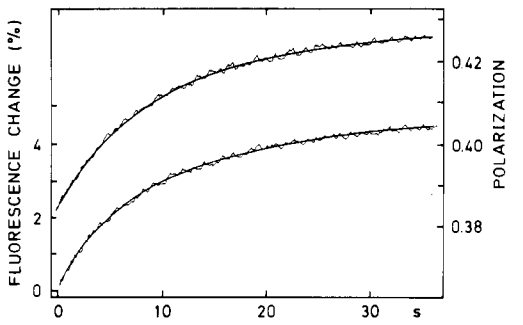

FIGURE 2: Ternary complex formation in the presence of IF-3. The stopped-flow experiment has been performed as described in Figure 1, except that IF-3 (1.8×10^{-7} M) was present in the ribosome solution. Titration experiments not shown here have revealed that an equimolar amount of IF-3 is sufficient to induce the maximal effect on the rate of complex formation. Both intensity (lower final level) and polarization (upper final level) of the fluorescence of proflavin have been measured. The changes of both observables are described by a single exponential function, yielding a relaxation time of 7 s. The fits obtained are indicated by the noiseless lines.

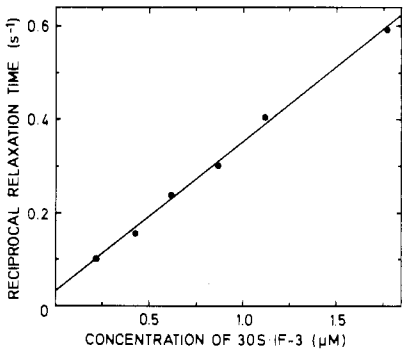

FIGURE 3: Dependence of the rate of ternary complex formation upon the concentration of 30S–IF-3. The kinetics of ternary complex formation have been measured as in Figure 2, except that the concentration of 30S subunits (and concomitantly of IF-3) was varied as indicated.

The rate constants for the formation and dissociation of the ternary complex have been determined from the dependence of the reciprocal relaxation times upon ribosome concentration. The linear dependence (Figure 3) indicates a second-order reaction, the rate constants of which are obtained from the slope ($k_{12} = 3 \times 10^5$ M^{-1} s^{-1}) and the ordinate intercept ($k_{21} = 0.03$ s^{-1}) of the plot (Bernasconi, 1976).

The rate of complex formation, measured as in Figure 2, does not change upon variation of the temperature between 10 and 30 °C (data not shown). This is in contrast to what has been observed by the filtration assay where the rate of complex formation had been found to be temperature dependent (Gualerzi et al., 1977, 1979). By this criterion, different steps, both taking place in the time range of seconds, appear to be monitored by the fluorescence and the filtration measurement, respectively.

Effects of Various Combinations of Initiation Factors and of GTP. The addition of the other two initiation factors and GTP further enhances the rate of ternary complex formation. Furthermore, in the complete system the formation of the complex is accompanied by a biphasic change of both

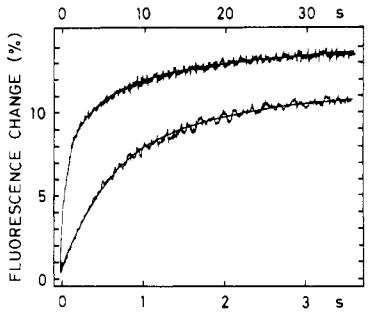

FIGURE 4: Ternary complex formation in the presence of the three initiation factors and of GTP. The stopped-flow experiment has been performed at 8 mM Mg^{2+} with *N*-AcPhe-$tRNA^{Phe}_{Prf37}$ (2×10^{-8} M) and poly(U)-programmed 30S ribosomal subunits (1.8×10^{-7} M) in the presence of IF-1, IF-2, IF-3 (all 1.8×10^{-7} M), and 0.5 mM GTP. Evaluation by least-squares fitting shows that the reaction progress curve has to be described by two-exponential terms characterized by the following relaxation times and relative amplitudes: $\tau_1 = 0.5$ s, $A_1 = 50\%$; $\tau_2 = 12$ s, $A_2 = 50\%$. The lower and upper time scales apply for the lower and upper curves, respectively.

Table I: Effect of Initiation Factors on the Rate of Ternary Complex Formation[a]

addition	relaxation time (s)[b] τ_1	τ_2
none	300	
IF-1	300	
IF-3	7.0	
IF-1, IF-3	4.0	
IF-2-GTP	4.5	11.5
IF-2-GTP, IF-3	2.2	14.0
IF-2-GTP, IF-1	1.0	4.5
IF-2-GTP, IF-1, IF-3	0.6	11.0

[a] The binding of *N*-AcPhe-$tRNA^{Phe}_{Prf37}$ to poly(U)-programmed 30S ribosomal subunits has been measured at 10 mM Mg^{2+}. Relaxation times have been obtained from the changes of both intensity and polarization of fluorescence as illustrated in Figure 2, except for the first two very slow reactions. In this case only polarization was measured. [b] ±20%.

fluorescence intensity (Figure 4) and polarization (data not shown), the two steps being characterized by relaxation times around 0.5 (τ_1) and 10 s (τ_2).

The effects of the individual initiation factors and GTP and of various combinations of them on the two relaxation times are summarized in Table I. The fast step will be considered first. The effect of IF-3 alone has already been discussed in the preceding paragraph. A comparable decrease of τ_1 (about 70-fold) is brought about by the addition of IF-2 together with GTP. The simultaneous presence of IF-2–GTP and IF-3 brings about a further decrease of τ_1 which is now approximately 150 times shorter than in the absence of factors. These data indicate that IF-2 and IF-3 do not act independently of each other in the sense that each of them brings about its full acceleration regardless whether the other factor is present or not (see Discussion). Unlike IF-2 and IF-3, IF-1 alone has no detectable effect on the rate of ternary complex formation. Only when IF-3 or IF-2–GTP or both are present, IF-1 produces a 2- or 4-fold increase, respectively, in the rate of complex formation.

In addition to the three initiation factors, GTP is required to get the full enhancement of ternary complex formation. Omission of GTP or its replacement with GDP results in an up to 10-fold increase of τ_1 in experiments with all three factors present or with IF-2 alone (not shown). Furthermore, in experiments without IF-2, the addition of GTP had no effect (data not shown). Thus, in line with previously published data, IF-2 appears to be the only target for the action of GTP.

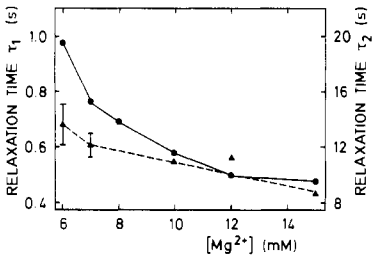

FIGURE 5: Dependence of the two relaxation times τ_1 and τ_2 observed in the complete system upon the concentration of Mg^{2+}. Experimental conditions as in Figure 4, except that the Mg^{2+} concentration was varied. The relaxation times τ_1 (●) and τ_2 (▲) were evaluated by two-exponential least-squares fitting of the kinetic curves.

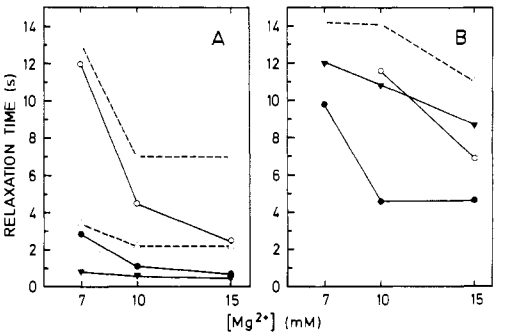

FIGURE 6: Mg^{2+} dependence of the relaxation times τ_1 (A) and τ_2 (B) observed in the presence of various combinations of initiation factors. See Figure 4 for experimental details. IF-3 (□); IF-2–GTP (○); IF-2–GTP–IF-3 (△); IF-1–IF-2–GTP (●); IF-1–IF-2–GTP–IF-3 (▼).

As to the slow step characterized by τ_2, Table I shows that it is observed only when IF-2 is present. This result strongly argues against the possibility that the slow step might be due to a heterogeneity of the ribosomal subunits such as the one reported previously (Blumberg et al., 1974). Nevertheless, in order to assess whether or not ribosome heterogeneity could account for the slow step observed, we compared 30S ribosomal subunits subjected to two additional high-salt washing cycles and subunits not washed at all. As described under Materials and Methods, these preparations differed in their estimated content of protein S1 (0.3 vs. 1 copy/particle on the average) and also in their relative content of other ribosomal proteins, e.g., S2, as compared to S4. In spite of this variation in protein content, no difference in both relaxation times and amplitudes could be detected in stopped-flow experiments with all three factors present. This result strongly suggests that the slow relaxation, τ_2, is not due to some heterogeneity of the ribosome preparation but, instead, is a genuine IF-2-induced reaction.

IF-1 decreases τ_2 about 2–3-fold, the same stimulation as observed for τ_1. Interestingly, IF-3 slightly inhibits the slow reaction, regardless whether IF-2 alone or both IF-2 and IF-1 are present. This observation strengthens the point made above that IF-2 and IF-3 do not act independently of each other.

Influence of Mg^{2+} on the Rate of Ternary Complex Formation. It has been mentioned above that the rate of ternary complex formation depends upon the Mg^{2+} concentration when no factor is present. The same is true in the presence of the factors, although there are considerable quantitative differences. In the complete system, i.e., in the presence of the three factors and GTP, τ_1 decreases by a factor of 2 when the Mg^{2+} concentration is varied from 6 to 15 mM; τ_2 changes somewhat less (Figure 5). Similarly, complex formation with only IF-3 present proceeds roughly 2 times faster at 15 as compared to 7 mM Mg^{2+} (Figure 6). An appreciably greater variation

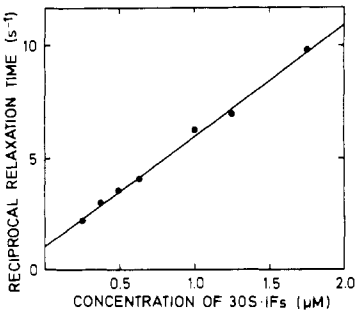

FIGURE 7: Dependence of the reciprocal relaxation time $1/\tau_1$ upon the concentration of 30S ribosomal subunits. The kinetics of ternary complex formation have been measured as in Figure 4 with varying concentrations of 30S subunits in the presence of equimolar concentrations of the three initiation factors.

is observed with IF-2–GTP, τ_1 decreasing about 6-fold in the same Mg^{2+} interval, and τ_2 even more. Upon addition of IF-1 together with IF-2–GTP, the effect of Mg^{2+} is reduced. Finally, upon completion of the system by addition of IF-3, the remaining effect of Mg^{2+} is rather small. The comparisons made in Table I, where data obtained at 10 mM Mg^{2+} have been presented, have to be qualified correspondingly.

It is interesting to note that both IF-1 and IF-3 exert their largest effects at the lowest Mg^{2+} concentration tested, 7 mM, which is close to the physiological conditions. At nonphysiological conditions, 15 mM Mg^{2+} for instance, IF-3 seems to be completely dispensable, provided the other two factors are present, at least as far as the rate of complex formation is concerned.

Rate Constants in the Complete System. The two relaxation times, τ_1 and τ_2, have been measured at varying concentrations of 30S ribosomal subunits in the presence of the respective equimolar (i.e., saturating) concentrations of the three initiation factors and 0.5 mM GTP. The plot of the reciprocal relaxation times vs. ribosome concentration (Figure 7) shows a linear relationship for $1/\tau_1$; $1/\tau_2$ does not vary at all (not shown). These data suggest a two-step model of factor-dependent ternary complex formation in which in a second-order step an intermediate complex is formed which rearranges to the final complex in a first-order reaction. The rate constants of the forward and reverse reactions of the first step, as determined from Figure 7, are $k_{12} = 5 \times 10^6\ M^{-1}\ s^{-1}$ and $k_{21} = 1.4\ s^{-1}$, respectively. The constant value of $1/\tau_2$, $0.1\ s^{-1}$, represents the sum of the two rate constants of the presumed rearrangement step, this figure then being the upper limit of either k_{23} or, less likely, k_{32}.

Discussion

The experiments carried out in this study were aimed at investigating two specific problems: (a) what is the mechanism of 30S initiation complex formation, and (b) what is the role of the initiation factors and GTP in this process.

Concerning the first point, the present kinetic data resolve two steps occurring during ternary complex formation between *N*-AcPhe-tRNA and 30S–poly(U) in the presence of initiation factors and GTP. The slower of these two steps is seen only in the presence of IF-2 and is first order; the faster one is seen with all combinations of factors and shows apparent second-order kinetics. The second-order rate constants obtained for this step range from $3 \times 10^5\ M^{-1}\ s^{-1}$, observed in the presence of IF-3 alone, to $5 \times 10^6\ M^{-1}\ s^{-1}$, observed in the presence of all three factors and GTP.

Second-order rate constants which are smaller than expected for a diffusion-limited recombination step (about $10^8\ M^{-1}\ s^{-1}$) may be interpreted in two ways. One may assume a sterically difficult recombination step, which the initiation factors facilitate by affecting the orientation factor. Alternatively, one may decompose the second-order step into the diffusion-limited step, which leads to a rather unstable, not directly observable encounter complex, and a subsequent rearrangement. We prefer the second alternative, since it is the one by which the accelerating effect of the initiation factors is more easily rationalized in structural terms.

It should be added that an additional possibility, not considered so far, is that the rate of recombination may also be limited by a transition in one of the complex partners, tRNA or ribosome, preceding the binding step. Thus, the complete reaction scheme describing the system may involve at least two states of each complex partner which are linked such that the final ternary complex can be reached via alternative pathways. Since we cannot distinguish these possibilities on the basis of the present data, the following interpretation is more qualitative.

On the basis of the fluorescence stopped-flow experiments, it can be postulated that the transition from a preternary complex to the ternary complex in the presence of all three initiation factors entails at least one and possibly two rearrangement steps. An additional rearrangement is indicated by a comparison of the results presented here with those obtained by the filtration assay (Gualerzi et al., 1977, 1979). These experiments revealed the existence of a rate-determining step in ternary complex formation which is influenced by the initiation factors. This step, in contrast to those observed by fluorescence, was found to be strongly temperature dependent, indicating that different steps are observed by the two techniques. In conclusion, the available data suggest that the final 30S initiation complex is formed by a two- or possibly three-step transformation of a preternary complex (Gualerzi et al., 1977) generated by the encounter between the 30S–poly(U) complex and *N*-AcPhe-tRNA.

As to the role played by the initiation factors, the results of this paper allow for the general conclusion that the rate of the first resolved step of ternary complex formation is very strongly (about 400-fold) increased by the presence of a full set of factors and GTP. Furthermore, when the effects of the individual factors has been studied, it has been found that either IF-3 or IF-2 (in the presence of GTP) brings about a substantial acceleration of comparable size (30–70-fold, depending upon the Mg^{2+} concentration). In the presence of both IF-3 and IF-2–GTP, a further 2–3-fold acceleration is observed, indicating that the two factors act in a synergistic fashion. On the other hand, the accelerating effect of the combination of the two factors is by far smaller than expected if the two factors were acting independently of each other. This behavior is best explained by assuming that the factors influence the same basic reaction and that upon addition of the factors the system approaches a limiting maximum velocity set by an intrinsic property of the system or one of its components, e.g., 30S ribosomal subunits of tRNA.

The presumed rearrangement reaction may be related to the "channeling" of the tRNA molecule from its primary, noncoded binding position to its final site on the 30S ribosomal subunit. In this regard, it is interesting to note that the forward rate constant of ternary complex formation in the complete system is close to the one observed for the association of the codon triplet with $tRNA^{Phe}$ in the absence of ribosomes (Labuda & Pörschke, 1980). Although this similarity of the rate constants may be coincidental, it appears possible that the first resolved step in ternary complex formation is related to co-

don–anticodon interaction. The failure to observe the fluorescence changes accompanying ternary complex formation when mRNA was omitted (data not shown) may be pertinent in this context.

The accelerating effect of both IF-2 and IF-3 may be explained by a direct effect on ribosome-bound aminoacyl-tRNA or, alternatively, by an allosteric influence on the 30S ribosomal subunit. A survey of some of the known properties of the two factors may be useful to choose between the two alternatives. Both factors possess a specific binding site on the 30S ribosomal subunit (Grunberg-Manago, 1980; Gualerzi & Pon, 1981) to which they can bind independently of each other (R. T. Pawlik, C. L. Pon, and C. Gualerzi, unpublished results). The fact that IF-2 and IF-3 may be cross-linked in situ and that many ribosomal proteins are topographical neighbors of both factors (Bollen et al., 1975; Grunberg-Manago, 1980; Gualerzi & Pon, 1981; R. R. Traut, personal communication) strongly suggests that the two sites are close to each other.

Both factors, by virtue of their RNA-binding properties interact with aminoacyl-tRNA. In the case of IF-3, the interaction is nonspecific. Thus, an effect of IF-3 on the ribosomal subunit appears more likely than an effect on the aminoacyl-tRNA. A model of IF-3 functioning based on an allosteric effect on the conformational dynamics of the 30S ribosomal subunit has been proposed (Pon et al., 1982).

For IF-2 its, albeit weak, affinity for N-blocked aminoacyl-tRNA (Majumdar et al., 1976; Sundari et al., 1976; Leon et al., 1979; Petersen et al., 1979) might suggest a direct effect of the factor on the ribosome-bound *N*-AcPhe-tRNA. On the other hand, it has been shown that IF-2 binding does not increase the affinity of the nonprogrammed 30S ribosomal subunit for either *N*-AcPhe-tRNA^{Phe} or fMet-tRNA$_f^{Met}$ (Gualerzi & Pon, 1981; C. L. Pon and C. Gualerzi, unpublished results), making unlikely an appreciable binding interaction between IF-2 and N-blocked aminoacyl-tRNA on the ribosome. Thus, to us it appears more likely that also IF-2 exerts an allosteric effect on the 30S ribosomal subunit.

IF-1 seems to be a different case. It is interesting that this factor influences the rate of ternary complex formation only in conjunction with IF-2 or IF-3, although IF-1 does bind to the 30S ribosomal subunit in the absence of the other two factors (Gualerzi & Pon, 1981). This result indicates that the observed IF-1-induced acceleration is due to an influence that IF-1 exercises on IF-2 and IF-3 at the 30S level and not to a direct effect on either the ribosomal subunit or the tRNA. This interpretation is in line with the findings that the IF-1 effect is quantitatively different with IF-2 and IF-3, respectively, and that IF-1 has no effect on the affinity of the 30S ribosomal subunits for aminoacyl-tRNA (C. L. Pon and C. Gualerzi, unpublished results).

Finally, a comment should be made on the slow IF-2-dependent rearrangement step. The fact that this reaction is brought about only by IF-2 indicates that it may represent a functionally important step. On the other hand, a rate constant of 0.1 s^{-1} or smaller is not really compatible with the rates expected for physiological initiation complex formation. One has to keep in mind, however, that the present experiments have been carried out with a model system which also lacks 50S ribosomal subunits. Thus, a possible explanation that we consider with particular interest is that the slow step might represent an additional reorientation of the aminoacyl-tRNA, which normally requires the presence of the 50S ribosomal subunits and GTP hydrolysis in order to occur at a rate comparable to that of the first rearrangement step. Experiments aimed at resolving that problem are presently in progress.

Acknowledgments

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