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RELAXATION KINETICS OF *E. COLI* RIBOSOMES: EVIDENCE FOR THE REACTION OF 30S • IF_3 COMPLEX WITH 50S RIBOSOMAL SUBUNITS

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Addition of initiation factor IF3 to solutions of *E. coli* ribosomes dramatically alters their behavior in pressure-jump relaxation kinetic experiments in which 90° light-scattering is used to monitor the macromolecular reaction. The effect of IF3 on relaxation processes attributed to "tight" couples is strongly dependent on the Mg^{2+} concentration. At 2.5 mM Mg^{2+}, addition of 1 molar equivalent of IF3 decreases the relaxation amplitude by a factor of 3 relative to ribosome solutions without IF3. However, at 5.0 mM Mg^{2+}, addition of 1 molar equivalent of IF3 produces a marked increase in the relaxation amplitude, by a factor of 2–8 fold relative to ribosomes in the absence of IF3. IF3 has no effect on the relaxation process attributed to "loose" couples at 10 mM Mg^{2+}. While we are unable to propose a precise mechanism for IF3 action with the data on hand, our results require that the 30S • IF3 complex either reacts with the 50S subunit, forming a 70S • IF3 intermediate, or acts as a pool of reactive 30S subunit. Further kinetic evidence is required to distinguish between these possible pathways.

1. Introduction ‡

For some time now, we have been using pressure and temperature jump relaxation kinetic methods to study the interaction of *E. coli* ribosomal subunits [1–3]. Briefly, our interpretation is that the association process obeys the mechanism

$$30S + 50S \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} 70S^* \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} 70S, \qquad (1)$$

where 70S* is a conformer of the 70S ribosome. The 30S and 50S subunits combine with a forward rate constant, k_{12}, approaching that expected for a diffusion controlled encounter, followed by a slow conformational rearrangement. We report here some initial observations on the effect of initiation factor IF3 on this association process. It is found that IF3 alters the relaxation kinetic behavior of *E. coli* ribosomes in a manner that is inconsistent with the major prediction of the popular anti-association model of IF3 action [5–10], whereby IF3 binds to the free 30S subunit, rendering it incapable of further association, and thus shifting the ribosome-subunit equilibrium towards dissociation by Le Chatelier's principle. Instead, our observations require that the 30S-IF3 complex either reacts with 50S subunit or acts as a pool of reactive 30S subunit, with the possibility that a 70S-IF3 complex may play a role in the mechanism of IF3 action.

‡ A brief account of this work was presented at the 62nd Annual Meeting of The Federation of American Societies for Experimental Biology, Atlanta, Georgia, June 8, 1978. See J.B. Chaires, M-S. Tai, G. Kegeles and A.J. Wahba, Fed. Proc. 37 (1978) 1659.

2. Materials and methods

The ribosomes used in these studies correspond to Type I preparations described in detail previously [3].

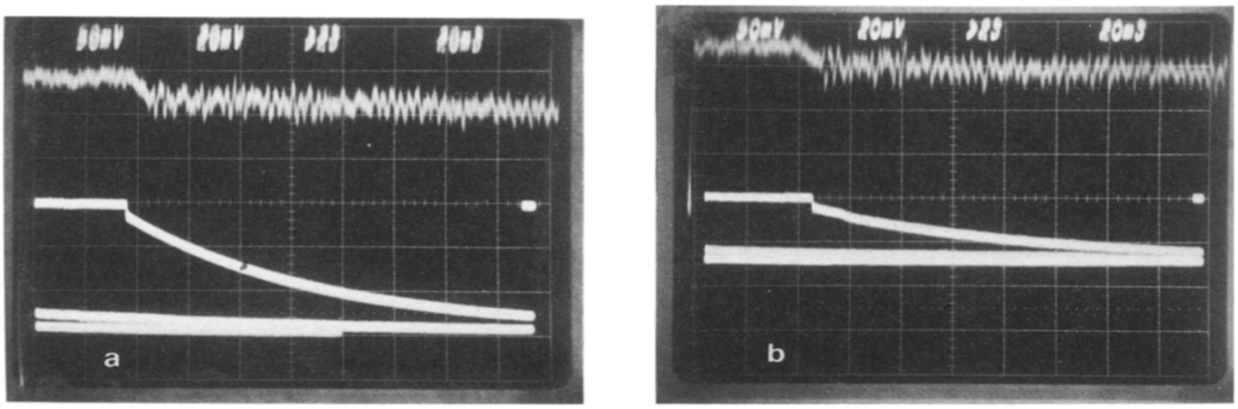

Fig. 1. Pressure jump relaxation patterns of *E. coli* ribosomes in buffer B, 2.5 mM Mg^{2+}. (A) 0.275% ribosome solution containing no IF3. The upper trace is scanned at 20 ms per horizontal division, while the lower is scanned at 7 s per division. For the top traces, the vertical sensitivity is 20 mV per division, while in the lower trace, the vertical sensitivity is 50 mV per division. (B) Identical to (A) with the addition of 1 molar equivalent of IF3.

Table 1
Summary of pressure-jump relaxation kinetic experiments

Experiment	RNP conc. (g/100 ml)	moles IF3 / moles RNP	N a)	Amplitude (mV)	τ (s)
A. 2.5 mM Mg^{2+}					
1	0.67	0	6	9.8 ± 2.4	15.6 ± 4.8
		1.0	5	5.0 ± 1.4	0.8 ± 0.1
2	0.57	0	5	21.2 ± 5.3	16.0 ± 3.9
		0.5	1	12.7 ± –	9.7 ± –
		1.0	5	7.3 ± 1.5	6.7 ± 1.9
3	0.54	0	5	19.9 ± 2.0	11.5 ± 1.6
		1.0	4	6.9 ± 3.8	1.9 ± 0.3
4	0.275	0	3	131.3 ± 0.6	28.4 ± 0.7
		1.0	4	55.6 ± 6.4	30.2 ± 1.3
B. 5.0 mM Mg^{2+}					
1	0.5	0	5	13.1 ± 2.3	12.6 ± 2.6
		1.0	5	41.8 ± 9.4	16.4 ± 3.5
2	0.25	0	4	9.1 ± 1.5	25.3 ± 9.6
		1.0	4	18.3 ± 3.2	34.2 ± 7.9
3	0.25	0	1	8.8 ± –	8.9 ± –
		1.0	3	19.9 ± 4.8	34.4 ± 13.1
4	0.275	0	2	11.3 ± –	19.2 ± –
		0.5	3	47.4 ± 1.3	29.2 ± 1.1
		1.0	3	85.4 ± 7.2	84.9 ± 9.5

a) N is the number of replicate experiments.

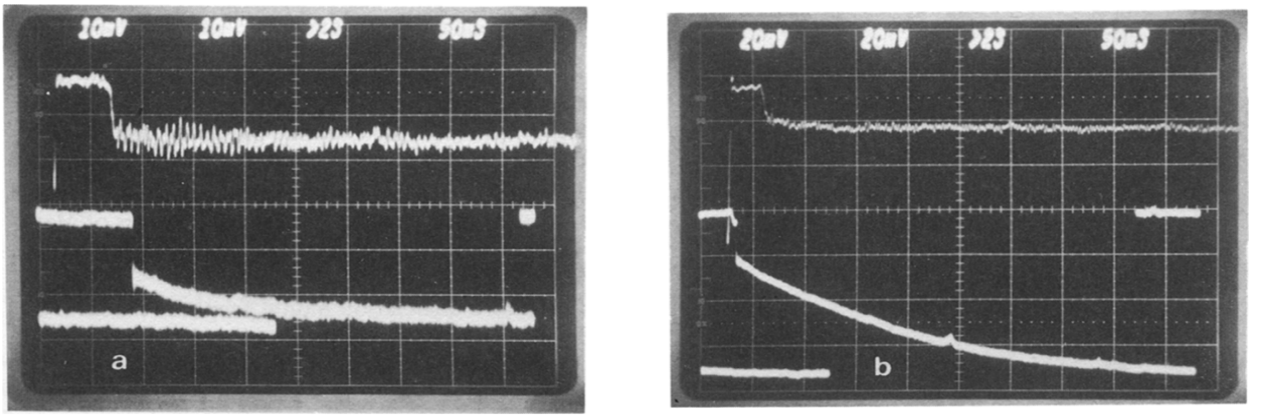

Fig. 2. Pressure jump relaxation patterns of *E. coli* ribosomes in buffer B, 5.0 mM Mg^{2+}. (A) 0.275% ribosome solution with no IF3. The upper trace is scanned at 50 ms per division while the lower is scanned at 7 s per division. Vertical sensitivity is 10 mV per division. (B) Identical solution as in (A), with the addition of 1.0 molar equivalents of IF3. The scanning speeds of the upper and lower traces are the same as in (A), but the vertical sensitivity is 20 mV per division.

Initiation factor IF3 was prepared according to the procedure described in [11]. The light-scattering pressure-jump equipment used in the present work has been extensively described [4]. All experiments were performed in a buffer ("Buffer B") consisting of 10 mM Tris-HCl pH 7.8, 60 mM KCl, 6 mM beta-mercaptoethanol, and $MgCl_2$ as indicated.

3. Results and discussion

Fig. 1 shows qualitatively the effect of IF3 on the pressure-jump relaxation pattern of *E. coli* ribosomes at 2.5 mM Mg^{2+}. A marked decrease in the relaxation amplitude is observed upon addition of IF3. The patterns shown represent the decrease in 90° light scattering of the ribosome solution following pressurization. Since IF3 has a molecular weight of only 22 000 daltons, its interaction with any ribosomal species would probably not be detected by 90° light-scattering, as will be discussed further in a later section. Thus, the patterns shown probably indicate only the consequences of IF3 addition on the ribosome-subunit interaction, without directly monitoring the interaction of IF3 with any ribosomal species. The effect of IF3 is seen more quantitatively in table 1, part A. In addition to the decrease in amplitude already ready noted, the relaxation time is also seen to decrease upon addition of IF3, although the small amplitude of the process makes it difficult to measure its precise value accurately. Further, the effect of IF3 on both the amplitude and relaxation time seems to be proportional to the amount of IF3 added, over the rather limited range of 0.0 to 1.0 molar equivalents of IF3.

At first sight, the effect of IF3 on the relaxation kinetic behavior at 5 mM Mg^{2+} appears to be different in nature. At 5 mM Mg^{2+}, addition of IF3, in comparison with control ribosomes with no IF3, increases both the relaxation amplitude and the relaxation time, as shown qualitatively in fig. 2, and more quantitatively in table 1, part B.

The relaxation patterns such as shown in figs. 1 and 2 all appear to be due to a single relaxation time, as judged by the linearity of plots of log (amplitude) versus time. As is seen in figs. 1 and 2, however, there is a sharp jump in amplitude in a time range beyond the resolution of our apparatus, which may represent a second relaxation process, as has been found for ribosomes in the absence of IF3 [3]. With the limited data on hand we are unable to interpret the observed relaxation times in terms of a precise mechanism for the cases in which IF3 has been added to the ribosome solution. Such interpretation requires more data on the concentration dependence of the relaxation time, and more critically, information on the rates of direct

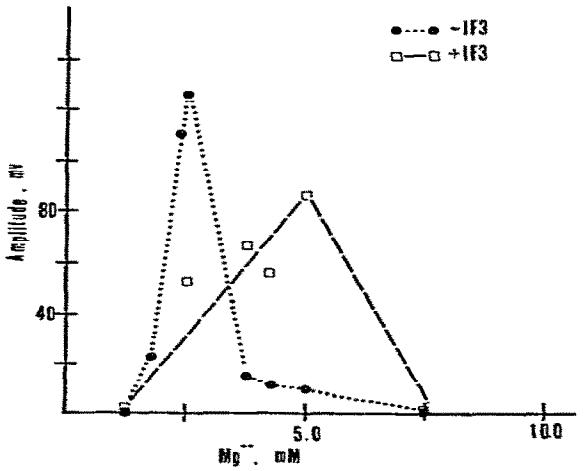

Fig. 3. Amplitude of slow decay portion of pressure jump relaxation curve, as a function of Mg^{2+}. The ribosome concentration was 0.275% in all cases, in Buffer B with Mg^{2+} as indicated. In experiments containing IF3, 1.0 molar equivalent was added.

IF3 interaction with the various ribosomal species.

The Mg^{2+} dependence of the relaxation amplitude is documented more fully in fig. 3. In the absence of IF3, the maximum amplitude is seen to occur near 2.5 mM Mg^{2+}, in agreement with our previous findings [3]. In the presence of IF3, the maximum is shifted to approximately 5 mM Mg^{2+}.

An explanation for the observed amplitude behavior is now sought. For a simple bimolecular association

$$30S + 50S \underset{k_r}{\overset{k_f}{\rightleftharpoons}} 70S \qquad (2)$$

the relaxation amplitude for a pressure-jump experiment is given by

$$A = \frac{\Delta V \Delta P}{RT}\left(\frac{1}{\overline{30S}} + \frac{1}{\overline{50S}} + \frac{1}{\overline{70S}}\right)^{-1}, \qquad (3)$$

where $\overline{30S}$, $\overline{50S}$, and $\overline{70S}$ are the equilibrium concentrations of the small subunit, large subunit, and ribosome, respectively, ΔV is the molar volume of reaction, ΔP is the magnitude of the pressure jump, R is the gas constant, and T is the absolute temperature. The same result obtains for reaction scheme (1) if 70S* is present at a small steady-state level, and ΔV is the volume change for the overall process. The amplitude is maximal when there is an approximately equimolar mixture of ribosomes and subunits, and the amplitude will become small if the concentration of 70S or either of the subunits becomes small. For example, the maximum amplitude in the absence of IF3 is seen in fig. 3 to occur at 2.5 mM Mg^{2+}, which is in good agreement with published values of $Mg^{2+}_{1/2}$ = 2.0–2.5 mM for *E. coli* "tight" couple subunit association [12,13].

The anti-association model proposes that IF3 sequesters free 30S subunits and blocks them from further association with 50S subunits:

$$70S \rightleftharpoons 50S + 30S$$
$$+$$
$$IF3$$
$$\updownarrow$$
$$30S \cdot IF3$$

The qualitative effect on this on the relaxation pattern would be to *decrease* the concentration of reactive 30S, and thus would *decrease* the relaxation amplitude while *increasing* the relaxation time. Such behavior is not observed in any of our experiments.

At 2.5 mM Mg^{2+} (fig. 1 and table 1, A) the relaxation amplitude decreases upon addition of IF3, in accord with the anti-association model, but the relaxation time is *decreased*, in contrast with the predictions of the model. It might be argued, in accordance with the anti-association model, that this behavior is due to the inhibition of the relaxation attributed to subunit association, and the superposed appearance of an IF3–subunit interaction. In this case, figure 1A would be interpreted to represent the ribosome–subunit interaction in the absence of IF3, and fig. 1B would represent an IF3–30S subunit interaction. However, addition of IF3 to the 30S subunit would change the molecular weight of that particle by only 2%, and, at less than 100% conversion of 30S subunit to the IF3 complex, this would have even less effect on the weight average molecular weight that we are monitoring with our light scattering method. Specifically, assuming a $K_a = 10^7\ M^{-1}$ for the reaction $30S + IF3 \rightleftharpoons 30S \cdot IF3$ a weight average molecular weight of 9.104×10^5 may be calculated assuming intial $[30S]_0$ = initial $[IF3]_0 = 10^{-6}$ M. If the reaction

Table 2
Calculated relaxation amplitude factors for assumes models of IF3 action

Reaction	K_{eq} (M^{-1})	Mg^{2+} (mM)	r (M)
30S + 50S ⇌ 70S	4.7×10^7	2.5	6.54×10^{-8}
30S + 50S ⇌ 70S	1.0×10^9	5.0	1.53×10^{-8}
30S + 50S ⇌ 70S [a]	1.0×10^9	5.0	2.2×10^{-9}
30S • IF3 + 50S ⇌ 70S • IF3	?	5.0	4.26×10^{-8}
30S • IF3 + 50S ⇌ 70S + IF3	100	5.0	4.12×10^{-8}
30S • IF3 + 50S ⇌ 70S + IF3	1	2.5	1.25×10^{-7}

[a] In the presence of IF3 which has sequestered a considerable amount of 30S.

were shifted 10% towards complex formation, as would be expected for a typical relaxation experiment, the weight average molecular shift would be changed by only 0.3%, to 9.134×10^5. In contrast, a comparable shift of the ribosome-subunit equilibrium would produce an approximate change in the weight average molecular weight from 2.14×10^6 to 2.22×10^6, or approximately 3.8% increase. Thus, by light scattering detection, we might expect that the observable amplitude for the 30S–IF3 interaction would be less than one tenth that observable for the ribosome-subunit interaction at 2.5 mM Mg^{2+} in the absence of IF3. On the contrary, the amplitude shown in fig. 1B is about one third of that in fig. 1A (see also part A, table 1). Since it is unlikely that we would detect direct IF3 interaction, the phenomenon shown in fig. 1B still remains to be explained.

At 5 mM Mg^{2+}, the increase in the observed relaxation time appears consistent with the anti-association model, but the observed increase in amplitude cannot be accounted for. Table 2 shows this quantiatively. Equilibrium concentrations of 30S, 50S, 70S, IF3 and 30S-IF3 were calculated for the coupled reactions

$$30S + 50S \rightleftharpoons 70S \qquad K_1 = \frac{[70S]}{[30S]\,[50S]}$$

$$30S + IF3 \rightleftharpoons 30S \cdot IF3 \qquad K_2 = \frac{[30S \cdot IF3]}{[30S]\,[IF3]}$$

at 5 mM Mg^{2+} using published values for the equilibrium constants of $K_1 = 10^9\ M^{-1}$ [13] and $K_2 = 10^7\ M^{-1}$ [7,10,14,15]. Comparison of lines 2 and 3 of table 2 shows that this model, which excludes reaction of the 30S-IF3 complex with the 50S subunit, predicts that IF3 should reduce the relaxation amplitude by a factor of seven, in marked contrast to our observed severalfold *increase* in relaxation amplitude upon IF3 addition at 5 mM Mg^{2+} (table 1B).

On the other hand, we might assume that the 30S-IF3 complex is reactive, for the reaction scheme

$$\begin{array}{ccc} \text{(I)} & & \text{(II)} \\ 70S & \underset{K_1}{\rightleftarrows} & 50S + 30S \\ + & & + \\ IF3 & & IF3 \\ K_4 \Big\updownarrow & & \Big\updownarrow K_2 \\ 70S \cdot IF3 & & 30S \cdot IF3 + 50S \\ \text{(IV)} & \underset{}{\overset{K_3}{\rightleftarrows}} & \text{(III)} \end{array} \qquad (4)$$

where

$K_1 = [70S]/[50S]\,[30S]$,

$K_2 = [30S \cdot IF3]/[30S]\,[IF3]$,

$K_3 = [70S \cdot IF3]/[30S \cdot IF3]\,[50S]$,

$K_4 = [70S]\,[IF3]/[70S \cdot IF3]$.

The equilibrium constants K_1 and K_2 have been measured at 5.0 mM Mg^{2+}, as mentioned before. At 2.5 mM Mg^{2+}, $1/K_4$ has been reported to be zero (i.e. no binding of IF3 to 70S particles), while at 5.3 mM Mg^{2+}, $1/K_4 = 6 \times 10^5 - 1.0 \times 10^7\ M^{-1}$ [10]. The value at 5.3 mM Mg^{2+} has been attributed to the interaction of IF3 with "loose" couples. Insufficient experimental details are given in [10] to critically evaluate the reliability of these numbers. Indeed, we fail to see how the authors were able to determine three independent unknown quantities (corresponding to our K_1, K_2 and $1/K_4$) from the single experimentally determined value $\bar{M}_w$.

Regardless, the overall equilibrium constant for the formation of 70S + IF3 from 30S • IF3 and 50S is K_3K_4. The product K_3K_4 is, however, by conservation of free energy, well known: it is necessarily equal to K_1/K_2 or approximately 100, at 5 mM Mg^{2+}. We might assume two extreme situations: a) K_4 is very small, and almost all of the 70S is in the form of an IF3 complex, or b) K_4 is very large and the 70S-IF3 complex is at a small steady state level during the pressure jump kinetics experiments. In the later case (b), the concentration-dependent factor in the overall relaxation amplitude can be calculated from eq. (5)

$$\Gamma = \left(\frac{1}{30S\text{-}IF3} + \frac{1}{50S} + \frac{1}{70S} + \frac{1}{IF3}\right)^{-1}. \qquad (5)$$

using $K_3K_4 = 100$ to obtain the concentration of species. We note here that the amplitude for the overall reaction from state III to state I is again given by eq. (5) if 30S is at a small steady state level and the reaction proceeds from state III to state I via state II; i.e. 30S • IF3 is simply a pool for reactive 30S. In case (a) we assume that 70S-IF3 represents all of the 70S concentration, and the amplitude is given by eq. (3). We calculate for case (a) in line 4 of table 2 a relative amplitude, Γ, some 2–4 times *larger* than that of ribosomes in the absence of IF3, assuming an identical $\Delta V \Delta P$ factor, in excellent agreement with our experimentally observed increases shown in table 1, part B. In line 5 of table 2 is shown the overall amplitude factor Γ for case (b) calculated from eq. (5), again in agreement with our experimental observations. Thus, our results are in accord with the requirement that the 30S-IF3 complex is in fact reactive, in contrast to the assertions of the anti-association model. Based on our results at 5 mM Mg^{2+}, however, we have no way to assess the stability of the presumed 70S-IF3 complex, or even the primary reaction pathway by which 30S • IF3 + 50S yield 70S + IF3. It should be emphasized here that equilibrium measurements, such as occur in the literature [5,6, 14,15] are intrinsically incapable of choosing between kinetic pathways.

Published kinetic studies report that IF3 decreases the overall forward rate constant k_f for ribosome subunit association, while the dissociation rate constant is unaltered [10,21,22]. Such behavior is expected if the anti-association model is correct. On the other hand, even in the absence of IF3 the forward rate constant reported in these studies is an *overall* rate constant with reference to mechanism (1) defined by

$$k_f = k_{12}k_{23}/(k_{21} + k_{23}),$$

while the overall dissociation rate constant is approximately given by

$$k_r \approx k_{32},$$

Mechanistic interpretations based on these overall rate constants thus become difficult. IF3 may be binding to the intermediate 70S* species, for example, which would lend to radically different interpretations of its mechanism of action.

Our results at 5.0 mM Mg^{2+}, in contrast to the interpretation of Godefroy-Colburn et al. [10], are attributed to the interaction of IF3 with "tight" couples. Sedimentation velocity experiments at 5.0 mM Mg^{2+} show, for the samples used in the experiments described in table 1B, a 70S zone with a trace of 50S material. No 30S material is evident. Thus, these samples seem to be largely free of "loose" couples. Further, relaxation kinetic experiments at 10 mM Mg^{2+}, where "loose" couples appear to exhibit maximum reaction amplitude when free of IF3 (3,19) show that IF3 has no effect on the relaxation process attributed to "loose" couples. This is documented in table 3.

Returning to the situation at 2.5 mM Mg^{2+}, we have attempted to explain the results in fig. 1A and 1B by the reaction scheme [4]. If it is assumed that $K_1 = K_2 = 10^7\ M^{-1}$, so that $K_3K_4 = 1$, then it is predicted from eq. (5) that the relative amplitude Γ is 1.25×10^{-7}, as shown in the last line of table 2, compared to 6.54×10^{-8}, table 2, line 1, for ribosomes at 2.5 mM Mg^{2+} in the absence of IF3. This prediction of a two-fold *increase* in amplitude upon addition of 1 copy of IF3 is to be contrasted with the experimental finding of a three-fold *decrease* in amplitude (table 1, part A), leading to a prediction that the 30S • IF3 formation constant at 2.5 mM Mg^{2+} is about one order of magnitude different from that at 5 mM Mg^{2+}.

The existence of a 70S • IF3 complex has been suggested by cross-linking experiments [16,20], electrophoretic studies [17], and inferred from chemical modification studies using lactoperoxidase [18]. How these studies, and the inference of a 70S-IF3 complex from our kinetic studies reported here, may be recon-

Table 3
Summary of pressure-jump experiments at 10 mM Mg^{2+} for the relaxation process attributed to "loose" couples

Experiment	RNP conc. (g/100 ml)	moles IF3 / moles RNP	N [a]	Amplitude (mV)	τ (ms)
1	0.23	0	4	12.6 ± 2.5	23.6 ± 1.3
		0.75	1	15.6	26.3
2	0.5	0	4	11.5 ± 1.0	26.7 ± 7.1
		0.75	10	10.6 ± 2.2	34.0 ± 7.4
3	0.8	0	5	12.5 ± 7.3	16.1 ± 3.7
		0.75	5	18.2 ± 8.2	19.4 ± 1.2
4	1.0	0	2	10.3 ± 0.9	29.7 ± 0.6
		0.75	6	13.1 ± 1.6	22.8 ± 3.8

[a] N is the number of replicate experiments.

ciled with failures to isolate such a complex using sucrose density gradient sedimentation [7–9] requires some explanation. One of us has shown [19] that, for the experimental conditions used in attempts to isolate the 70S-IF3 complex, an association constant of 10^7 M^{-1} is required (in the absence of any pressure effects) in order to detect complex formation. Thus, one only needs to postulate a complex with a formation constant lower than this to reconcile the apparent contradiction. Alternatively, the proposed 70S-IF3 complex might be more readily dissociated by pressure than are 70S ribosomes alone, making it extremely difficult to isolate the 70S-IF3 complex using centrifugation. Such an increased sensitivity to pressure might also account for our observed increases in relaxation amplitude at 5 mM Mg^{2+}, although this postulate is not necessary to account for our observations. While the postulate of a reactive 30S-IF3 complex can account superficially for some of our observations, we wish to emphasize that we are unable at this point to specify a detailed mechanism of IF3 action with the data on hand. The relaxation times shown in figs. 1 and 2 and table 1 are too slow to represent elementary, diffusion controlled steps, and must represent composite processes. In particular, the initial interaction of IF3 with either subunits or ribosomes should occur at a rate near diffusion control, but with 90° light-scattering observation, we have not monitored this step in the current experiments. Such a step must be included in any detailed mechanism. Further, any proposed mechanism should account for the clear Mg^{2+} dependence observed in the experiments reported here. Finally, we have not considered the efect of our previous finding that the association of subunits is a two step process. IF3 may react with 70S* and not 70S, for example, leading to a considerably more complicated mechanism that discussed here. Nevertheless, the postulate of a reactive 30S-IF3 complex at 5 mM Mg^{2+} seems inescapable from the relaxation amplitude data we gave gathered thus far, and must be considered in any detailed mechanism that emerges from further study.

4. Summary

Based on our consideration of relaxation amplitudes, we conclude that the 30S • IF3 complex must be reactive, either reacting directly with the 50S subunit to form a 70S • IF3 complex, or acting as a pool of reactive 30S subunits. Published equilibrium studies are intrinsically incapable of distinguishing between these pathways, necessitating a kinetic approach to solve the problem. However, published kinetic studies report only on the effect of IF3 on the ribosome subunit equilibrium *overall* rate constants, and thus may neglect the interaction of IF3 with an important ribosomal intermediate. While we have insufficient kinetic data at this time to propose a precise mechanism for IF3 action, the relaxation kinetic methods we present here should offer a fruitful approach to solving the problem.

Acknowledgement

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Note added in proof: Preliminary experiments indicate that dilution of a concentrated ribosome stock solution at 16 mM Mg^{2+} into buffer at 2.5–5.0 mM Mg^{2+} to form a 0.25% ribosome solution results in a free Mg^{2+} concentration approximately 20% *lower* than that expected from the composition of the buffers alone, due to some change in the binding of Mg^{2+} by the ribosome. The presence of IF3 during such a dilution appears to cause a smaller diminuation of Mg^{2+} relative to ribosome solutions without IF3. This shifts the values on the abscissa in fig. 3 to the right, but does not qualitatively affect the arguments made in this paper. This dilution procedure has also been used in other studies [3,23,24], since dialysis to control carefully the Mg^{2+} concentration has been found to lead to "loose" couple formation [3,24].

References

[1] J.B. Chaires, C. Ke, M.-S. Tai, G. Kegeles, A.A. Infante, B. Kosiba and T. Coker, Biophys. J. 15 (1975) 293a.
[2] J.B. Chaires and G. Kegeles, Biophys. Chem. 7 (1977) 173.
[3] J.B. Chaires, M. Tai, C. Huang, G. Kegeles, A.A. Infante and A.J. Wahba, Biophys. Chem. 7 (1977) 179.
[4] G. Kegeles and C. Ke, Anal. Biochem. 68 (1975) 138.
[5] R. Kaempfer, in: Ribosomes, eds. M. Nomura, A. Tissieres and P. Lengyel (Cold Spring Harbor monograph series, Cold Spring Harbor, N.Y., 1974) pp. 679–704.
[6] B.D. Davis, ibid, pp. 705–710.
[7] S. Sabol, D. Meier and S. Ochoa, Eur. J. Biochem. 33 (1973) 332.
[8] C. Gualerzi and C.L. Pon, BBRC 52 (1973) 792.
[9] J. Thibault, A. Chestier, D. Victul and F. Gros, Biochimie 54 (1972) 829.
[10] T. Godefroy-Colburn, A.D. Wolfe, J. Dondon, M. Grunberg-Manago, Ph. Dessen and D. Pantaloni, J. Mol. Biol. 94 (1975) 461.
[11] N. Shiff, M.J. Miller and A.J. Wahba, J. Biol. Chem. 249 (1974) 3797.
[12] P. Debey, G. Hui Bon Hoa, P. Douzou, T. Godefroy-Colburn, M. Graffe and M. Grunberg-Manago, Biochemistry 14 (1975) 1553.
[13] M. Noll and H. Noll, J. Mol. Biol. 105 (1975) 111.
[14] A. Subramanian and B.D. Davis, Nature 228 (1970) 1273.
[15] J. Weil, J.W.B. Hershey and S.A. Levison, FEBS Lett. 87 (1978) 103.
[16] D.A. Hawley, M.J. Miller, L.I. Slobin and A.J. Wahba, BBRC 61 (1974) 329.
[17] A. Talens, O. van Diggelen, M. Bronyers, L.M. Popa and L. Bosch, Eur. J. Biochem. 37 (1973) 121.
[18] C. Michalski, B. Sells, R. Chowdhury and A.J. Wahba, Eur. J. Biochem. 89 (1978) 589.
[19] J.B. Chaires, Ph.D. Thesis, University of Connecticut, U.S.A. (1978).
[20] C. Vermeer, J. Boon, A. Talens and L. Bosch, Eur. J. Biochem. 40 (1974) 283.
[21] M. Gottlieb, B.D. Davis and R.C. Thompson, Proc. Nat. Acad. Sci. U.S.A. 72 (1975) 4238.
[22] M. Noll, Ph.D. Thesis, Northwestern University, U.S.A. (1972)
[23] A. Wishnia, A. Boussert, M. Graffe, Ph. Dessen and M. Grunberg-Manago, J. Mol. Biol. 93 (1975) 499.
[24] H. Görish, D.J. Goss and L.J. Parkhurst, Biochemistry 14 (1976) 5743.