the attempts to decompose our spectra into the two Lorentzian components by use of eq A-1 was successful.

References

Berne, B. J., and Pecora, R. (1976), Dynamic Light Scattering, New York, N.Y., Wiley.

Blasie, J. K. (1972), *Biophys. J. 12,* 205.

Busch, G. E., Applebury, M. L., Lamola, A. A., and Rentzepis, P. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 2802.

Chu, B. (1974), Laser Light Scattering, New York, N.Y., Academic Press.

Cummins, H. Z., and Swinney, H. L. (1970), in Progress in Optics, Vol. VIII, Walf, E., Ed., Amsterdam, North-Holland Publishing Co.

Draper, N. R., and Smith, H. (1966), Applied Regression Analysis, New York, N.Y., Wiley.

Hagins, W. A. (1972), *Annu. Rev. Biophys. Bioeng. 1,* 131.

Hagins, W. A., and Yoshikami, S. (1974), *Exp. Eye Res. 18,* 299.

Han, C. C.-C., and Yu, H. (1974), *J. Chem. Phys. 61,* 2650.

Heller, J., Ostwald, T. J., and Bok, D. (1970), *Biochemistry 9,* 4884.

Hendriks, Th., Daemen, F. J. M., and Bonting, S. L. (1974), *Biochim. Biophys. Acta 345,* 468.

Mason, W. T., Fager, R. S., and Abrahamson, E. W. (1974), *Nature (London) 247,* 562.

McConnell, D. G. (1975), *J. Biol. Chem. 250,* 1898.

Norisuye, T., Hoffman, W. F., and Yu, H. (1976), *Biochemistry 15,* 5678.

Oseroff, A. R., and Callender, R. H. (1974), *Biochemistry 13,* 4243.

Pecora, R. (1972), *Annu. Rev. Biophys. Bioeng. 1,* 257.

Poo, M. M., and Cone, R. A. (1973), *Exp. Eye Res. 17,* 503.

Raubach, R. A., Nemes, P. P., and Dratz, E. A. (1974), *Exp. Eye Res. 18,* 1.

Schurr, J. M., and Schmitz, K. S. (1973), *Biopolymers 12,* 1021.

Shaya, S. A., Han, C. C.-C., and Yu, H. (1974), *Rev. Sci. Instrum. 47,* 280.

Smith, Jr., H. G., Stubbs, G. W., and Litman, B. J. (1975), *Exp. Eye Res. 20,* 211.

Wada, A., Suda, N., Tsuda, T., and Soda, K. (1969), *J. Chem. Phys. 50,* 31.

Weller, M., Virmaux, N., and Mandel, P. (1975), *Nature (London) 256,* 68.

Worthington, C. R. (1973), *Exp. Eye Res. 17,* 487.

Yoshizawa, T., and Wald, G. (1963), *Nature (London) 197,* 1279.

Stabilization by the 30S Ribosomal Subunit of the Interaction of 50S Subunits with Elongation Factor G and Guanine Nucleotide†

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ABSTRACT: The role of the 30S ribosomal subunit in the formation of the complex ribosome·guanine nucleotide·elongation factor G (EF-G) has been examined in a great variety of experimental conditions. Our results show that at a large molar excess of EF-G or high concentrations of GTP or GDP, 50S ribosomal subunits are as active alone as with 30S subunits in the formation of the complex, while at lower concentrations of nucleotide or lower amounts of EF-G, addition of the 30S subunit stimulates greatly the reaction. The presence of the 30S ribosomal subunit can also moderate the inhibition of the 50S subunit activity that occurs by increasing moderately the concentrations of K^+ and NH_4^+, and extends upward the concentration range of these monovalent cations in which complex formation is at maximum. The Mg^{2+} requirement for complex formation with the 50S subunit appears to be slightly less than that needed for association of the 30S and 50S ribosomal subunits. Measurement of the reaction rate constants of the complex formation shows that the 30S ribosomal subunit has only little effect on the initial association of EF-G and guanine nucleotide with the 50S subunit; but once this complex is formed, the 30S subunit increases its stability from 10- to 18-fold. It is concluded that stabilization of the interaction between EF-G and ribosome is a major function of the 30S subunit in the ribosome-EF-G GTPase reaction.

During protein synthesis, the interaction of the ribosome with elongation factor G (EF-G)[1] and the accompanying hydrolysis of GTP result in the translocation of peptidyl-tRNA from the ribosomal acceptor to donor site and movement relative to the mRNA (for review, see Haselkorn and Rothman-Denes, 1973). At low concentration of the monovalent cations K^+ and NH_4^+, the 50S ribosomal subunit is fully capable of supporting a high level of EF-G-dependent GTP hydrolysis; but at higher, more physiological concentrations, the 30S ribosomal subunit is required (Voigt et al., 1974; Parmeggiani et al., 1974; Arai and Kaziro, 1975). To gain insight into the manner in which the 30S ribosomal subunit is involved, we have examined its role in the formation of EF-G·guanine nucleotide·ribosome complex, which represents a stabilized intermediate of the GTPase reaction.

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[1] Abbreviations used: EF-G, elongation factor G; GMPPCP, 5′-guanylylmethylenediphosphonate; PEI, polyethylenimine; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane.

Complex formation can occur with the reaction product, GDP, or with the substrate GTP, in which case hydrolysis rapidly converts the nucleotide to GDP before the complex can be assayed by present techniques (Brot et al., 1969, 1971; Parmeggiani and Gottschalk, 1969; Bodley et al., 1969; Kuriki et al., 1970). The antibiotic fusidic acid stabilizes the EF-G·GDP·ribosome complex and therefore is widely used to facilitate its detection (Bodley et al., 1969, 1970). Alternatively, nonhydrolyzable analogues of GTP such as GMPPCP may be used to form a stable ternary complex (Parmeggiani and Gottschalk, 1969; Kuriki et al., 1970; Brot et al., 1971; Eckstein et al., 1975).

Some authors have reported that complex formation depends only upon the 50S ribosomal subunit (Bodley and Lin, 1970; Brot et al., 1971), while others have observed a need for or a large stimulation by the 30S subunit (Parmeggiani and Gottschalk, 1969; Hamel and Nakamoto, 1972; Sander et al., 1975). The present experiments explain these conflicting observations in terms of the experimental conditions used, viz. the guanine nucleotide and K^+ or NH_4^+ concentration and the molar excess of EF-G over 50S subunits, and show on the basis of kinetic studies that the primary function of the 30S subunit is to stabilize the EF-G·guanine nucleotide·ribosome complex once formed. Recently Arai and Kaziro (1975) have also reported that the 30S requirement for complex formation depends upon the NH_4^+ concentration.

Materials and Methods

Nucleotides. GDP and GTP were obtained from Boehringer, Mannheim, Germany, as Li^+ salts and converted to the NH_4^+ form by passage through a column of AG-50W-X2 cation-exchange resin (Bio-Rad Laboratories, Richmond, Calif.) previously washed with 1 M NH_4Cl. GMPPCP was obtained from Miles Laboratories, Elkhart, Ind. The tritium-labeled nucleotides were purchased as NH_4^+ salts from The Radiochemical Centre, Amersham, U.K. Purity of all nucleotides was checked by thin-layer chromatography on PEI-cellulose run in 0.75 M phosphate buffer (pH 3.5) or 1 M LiCl. When necessary, the unlabelled nucleotides were repurified by chromatography on a column of DEAE-cellulose in bicarbonate form. A gradient of triethylammonium bicarbonate (pH 7.5) was used for elution. Specific radioactivities were determined from absorbance at 260 nm and scintillation counting in toluene containing 6 g of 2,5-diphenyloxazole per L and were checked by isotopic dilution with the unlabeled nucleotides in the ternary complex assay.

EF-G and Ribosomes. Electrophoretically homogeneous EF-G was purified from *Escherichia coli* $BT2^r$ as described by Parmeggiani et al. (1971), except that Sephadex G-200 filtration was replaced by chromatography on DEAE-Sephadex A-50. EF-G was stored at −25 °C in 50 mM Tris-HCl (pH 7.8)–2 mM dithiothreitol containing 50% glycerol. Based on a M_r of 84 000, 1 μg of EF-G corresponds to 12 pmol (Parmeggiani and Gottschalk, 1969; Arai et al., 1973).

Ribosomes were isolated and washed with 0.5 M NH_4Cl as described by Sander et al. (1975). Subunits were prepared by centrifugation of the ribosomes at 0.5 mM $MgCl_2$ through a linear 5–25% sucrose gradient using a Spinco 15 Ti zonal rotor (Sander et al., 1975). The 30S subunits were at least 98% pure and the 50S subunits 95%, as judged by sedimentation in analytical sucrose gradients. They were stored at −25 °C in 20 mM Tris-HCl (pH 7.8)–10 mM $MgCl_2$–30 mM KCl–30 mM NH_4Cl containing 50% glycerol. One A_{260} unit was taken to correspond to 25 pmol of 70S, 39 pmol of 50S, or 67 pmol of 30S particles.

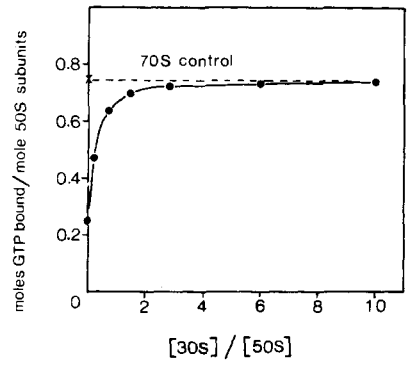

FIGURE 1: Dependence of EF-G·GTP·ribosome complex formation on the 30S to 50S ribosomal subunit ratio. [^{3}H]GTP binding was measured using the basic reaction mixture to which increasing amounts of 30S subunits were added.

Assay for Ternary Complex. The EF-G·guanine nucleotide·ribosome complex was measured by virtue of its ability to bind to nitrocellulose filters. The basic reaction mixture contained 3 pmol of 70S ribosomes or 50S subunits, plus or minus 15 pmol of 30S subunits, and 15 pmol of EF-G in 50 μL of 50 mM Tris-HCl (pH 7.8)–14 mM $MgCl_2$–10 mM NH_4Cl–14 mM 2-mercaptoethanol–3% glycerol–2 mM fusidic acid and 0.22 μM [^{3}H]GTP or [^{3}H]GDP (1–1.5 Ci/mmol). Depending on the parameter investigated the amounts of EF-G, GTP, GDP, $MgCl_2$, or NH_4Cl present in the different experiments were varied as indicated in the figures. To ready the nitrocellulose filters (Millipore HAWP, 2.4-cm diameter, 0.45-μm pore size), they were soaked in wash buffer consisting of 50 mM Tris-HCl (pH 7.8)–14 mM $MgCl_2$–10 mM NH_4Cl–14 mM 2-mercaptoethanol–0.1 mM fusidic acid, placed in a filtration apparatus, and overlaid with 3 mL of cold wash buffer. After the reaction mixture had been incubated at 30 °C for 20 min, a 40-μL sample was withdrawn and gently pipetted under the wash buffer onto the surface of the filter. Due to the small amount of glycerol in the reaction mixture, it quickly spread evenly over the filter without being diluted. Suction was then applied so that filtration and washing lasted only 1–2 s. After drying the filters, bound radioactivity was measured as above.

Results

Dependence of EF-G·Guanine Nucleotide·Ribosome Complex Formation on the 30S Ribosomal Subunit. At the relatively low levels of reactants chosen for the initial experiments (see basic reaction mixture in Materials and Methods), 50S subunits were only one third as active as 70S ribosomes in ternary complex formation with EF-G and GTP (Figure 1). Addition of 30S subunits to the 50S subunits strongly increased activity, with the 75% level of activity exhibited by the 70S ribosomes being approached at an equimolar ratio of the two subunits. Practically identical results were observed with GDP (not shown). By themselves, the 30S subunits showed no complex forming ability.

The values given represent the level of complex present after the reaction has reached equilibrium, so the lower activity of the 50S subunits was not due to a difference in the rate of complex formation.

Effect of Cation Concentrations on Complex Formation. In an attempt to reconcile the differences in the literature with respect to a 30S requirement for ternary complex formation, the response to Mg^{2+} and monovalent cation concentrations was examined. As shown in Figure 2, there was only a very

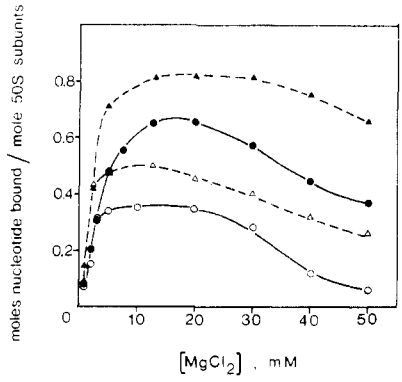

FIGURE 2: Effect of Mg^{2+} concentration on ternary complex formation with GDP (triangles) and GTP (circles). Assays were conducted with the basic reaction mixture and wash buffer containing different concentrations of $MgCl_2$. Open symbols represent 50S subunit activity without 30S subunits, filled symbols, 50S subunit activity with 30S subunits.

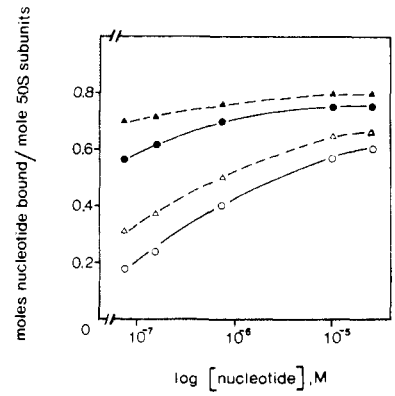

FIGURE 4: Dependence of ternary complex formation on GDP (triangles) or GTP (circles) concentration. Assays were conducted with the basic reaction mixture containing the indicated concentrations of ^3H-labeled nucleotide. Open symbols represent 50S subunit activity without 30S subunits, filled symbols, 50S activity with 30S subunits.

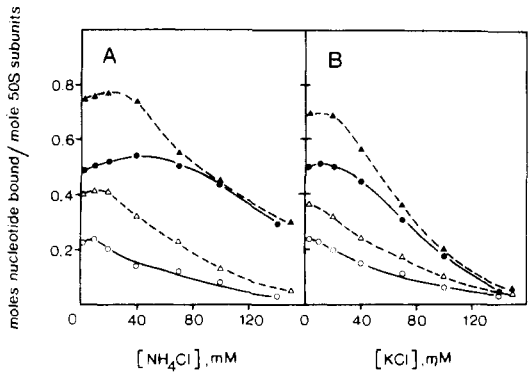

FIGURE 3: Effect of NH_4^+ (A) and K^+ (B) concentration on ternary complex formation with GDP (triangles) and GTP (circles). Assays were conducted with the basic reaction mixture and wash buffer containing different concentrations of NH_4Cl or KCl. With KCl, the 10 mM NH_4Cl normally present was omitted. Open symbols represent 50S subunit activity without 30S subunits, filled symbols, 50S subunit activity with 30S subunits.

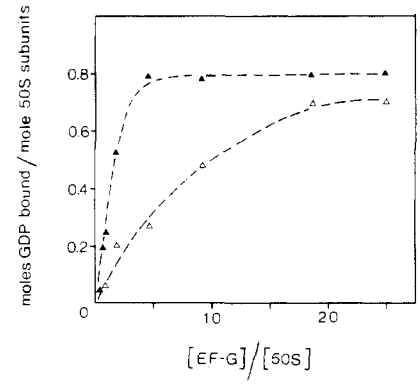

FIGURE 5: Dependence of ternary complex formation with GDP on the molar ratio of EF-G to 50S ribosomal subunits. Assays were conducted with the basic reaction mixture containing the indicated amounts of EF-G. Open symbols represent 50S subunit activity without 30S subunits, filled symbols, 50S subunit activity with 30S subunits.

narrow range in Mg^{2+} concentration (1-3 mM) where 50S subunit activity was not stimulated by the 30S subunits. Apparently, in this range the Mg^{2+} concentration was able to support ternary complex formation with the 50S subunits but not association of the 30S and 50S subunits. At the higher concentrations of Mg^{2+} tested, activity both with and without 30S subunits began to decline. A corresponding decrease in GTPase activity has also been described (Parmeggiani et al., 1974).

Over the full range of NH_4^+ (Figure 3A) concentration tested, the presence of 30S subunits stimulated ternary complex formation, with the relative degree of stimulation increasing as the concentration of monovalent cation was raised. This occurred because 50S subunit activity fell continuously from optima in the 1-20 mM range, whereas the presence of the 30S subunit extended the range for maximum activity to approximately 40 and 80 mM NH_4^+ with GDP and GTP, respectively.

In the presence of K^+ (Figure 3B), stimulation of complex formation by 30S subunit was also observed. However, the ratio of 50S subunit activity with and without 30S subunits changed little as a function of K^+ concentration.

Dependence of Complex Formation on Reactant Concentrations. Stimulation of 50S subunit activity was attempted by increasing the concentration of either guanine nucleotide or EF-G. As the concentration of GDP or GTP was raised from the 2.2×10^{-7} M level used in the previous experiments to 2.2×10^{-5} M, participation of the 50S subunits in complex formation doubled (Figure 4). At the same time activity in the presence of 30S subunits increased only moderately. Consequently, activity of the 50S subunits neared that found in the presence of added 30S subunits.

Much the same effect was observed when the molar ratio of EF-G to 50S subunits was increased (Figure 5). At an 18-fold excess of EF-G, participation of the 50S subunits in complex formation reached 70% with GDP. In the presence of 30S subunits, this level was reached with a 3-fold excess of EF-G. Analogous but slightly lower activity was seen with GTP (not shown).

These results make it clear that a wide variation in 50S vs. 50S + 30S activity can be experimentally obtained depending on both the cation and reactant concentrations chosen. They further indicate that the presence of the 30S subunit shifts the equilibrium between reactants and ternary complex in favor of the complex.

Kinetics of Ternary Complex Formation. The overall reaction for complex formation with GDP is

$$\text{EF-G} + \text{GDP} + \text{ribosome} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{EF-G·GDP·ribosome}$$

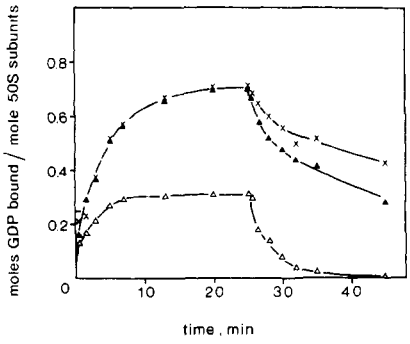

FIGURE 6: Time course of ternary complex formation and breakdown. The 1.6-mL reaction mixture contained the same buffer, salt, and fusidic acid concentrations as the basic reaction mixture along with 24 pmol of 70S ribosomes or 50S subunits, plus or minus 72 pmol of 30S subunits, 120 pmol of EF-G, and a [^{3}H]GDP concentration of 0.1 μM at 2 Ci/mmol. Before complex formation was initiated by adding [^{3}H]GDP, the mixture was incubated at 30 °C for 20 min, a time found sufficient to reactivate those ribosomal particles which had lost activity during storage. After the [^{3}H]GDP was added, incubation was continued at 30 °C and 100-μL samples were removed at intervals for assay. At the end of 25 min, the concentration of nucleotide was brought to 0.1 mM by addition of unlabeled GDP and the sampling continued to follow complex breakdown: (△) 50S subunit activity without 30S subunits; (▲) 50S subunit activity with 30S subunits; and (×) 70S ribosomal activity.

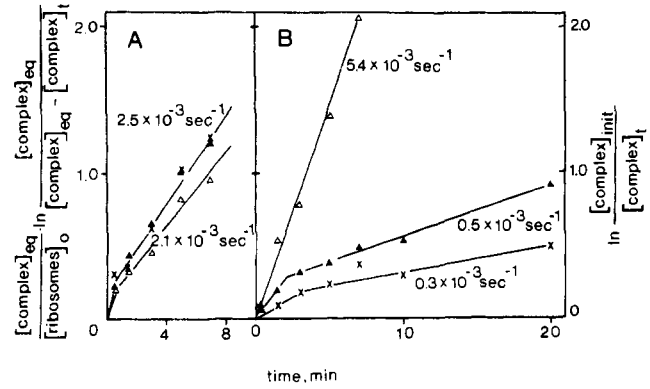

FIGURE 7: Determination of the apparent forward (A) and reverse (B) rate constants for complex formation with GDP. The data in Figure 6 have been replotted according to eq 1 in the case of complex formation and according to the equation which describes a unidirectional first-order reaction in the case of complex breakdown: $K_{-1}t = \ln([\text{complex}]_{init}/[\text{complex}]_t)$, where $[\text{complex}]_{init}$ and $[\text{complex}]_t$ are the concentrations of complex at the initiation of breakdown and time t, respectively. In plotting these curves, 80% of the 50S subunits were assumed to be active. This was based on maximum complex formation under optimal conditions. The rate constants are equal to the slopes of the curves and are given in the figure. Symbols as in Figure 6.

regardless of the number of intermediate steps. To simplify an analysis of the effect of 30S subunits on the rate constants of this reaction, an excess of EF-G and GDP over 50S, 70S, or 50S plus 30S ribosomal particles was used. Their concentrations, which remained relatively constant during the reaction, could thus be incorporated into the forward rate constant, and the forward reaction described in terms of pseudo-first-order kinetics. The time course of the overall reaction is then described by the general equation for reversible first-order reactions:

$$k_1't = \frac{[\text{complex}]_{eq}}{[\text{ribosomes}]_0} \ln \frac{[\text{complex}]_{eq}}{[\text{complex}]_{eq} - [\text{complex}]_t} \quad (1)$$

where $k_1' = k_1[\text{GDP}][\text{EF-G}]$, $[\text{complex}]_{eq}$ and $[\text{complex}]_t$ are the concentration of complex at equilibrium and time t, respectively, and $[\text{ribosomes}]_0$ is the initial concentration of 50S or 70S ribosomal particles.

The experimental time courses of complex formation with 70S ribosomes and 50S subunits, with and without 30S subunits, are shown in Figure 6. Under the dilute conditions employed, equilibrium was reached within 20 min in each case. Upon replotting the data according to eq 1, nearly identical pseudo-first-order rate constants were found for the majority of the 50S, 50S plus 30S, and 70S particles (Figure 7A). This indicates that the 30S subunits increase little the affinity of 50S subunits for EF-G and GDP.

Complex breakdown was determined by following the time course of the exchange reaction taking place in the presence of a 1000-fold excess of unlabeled GDP added to the reaction mixtures after equilibrium was established (Figure 6). With 50S subunits, breakdown proceeded very rapidly, whereas the complex formed with 70S ribosomes or 50S plus 30S subunits was relatively stable. As determined from a first-order plot of the data in Figure 7B, the k_{-1} of 5.4×10^{-3} s^{-1} for 50S subunits was 10 times higher than that found in the presence of 30S subunits and 18 times that with 70S ribosomes. Thus, the 30S subunit appears to affect the equilibrium of complex formation purely by stabilizing the complex once formed.

Reverse rate constants almost identical with those with GDP were found when the ternary complex was formed with GTP (data not shown). Hydrolysis of the GTP, therefore, does not affect complex stability at least in the presence of fusidic acid.

Because fusidic acid was present in the preceding experiments and may have influenced the action of the 30S subunit, breakdown of the complex formed with GDP in the absence of fusidic acid, as well as of the complex formed with GMPPCP was checked. In both cases the same conclusion with regard to the 30S subunit could be drawn. Readily measurable amounts of EF-G·GDP·50S subunit complex up to 0.1 mol of GDP/mol of 50S subunit were obtained in the absence of fusidic acid by increasing the concentration of all reactants 10- to 20-fold, but its breakdown was complete within 0.5 min of the addition of excess unlabeled GDP and proved too fast to be accurately followed by the filter paper assay. On the other hand, complex formed in the presence of 30S subunits or with 70S ribosomes was stable enough to allow the reverse rate constants to be determined. As might be expected, each k_{-1} value was 100-fold greater than when fusidic acid was present. With GMPPCP, the respective k_{-1} values with 50S, 50S plus 30S, and 70S particles were 21.7×10^{-3}, 3.7×10^{-3}, and 2.7×10^{-3} s^{-1}.

Discussion

Ternary complex formation between EF-G, guanine nucleotide, and ribosomes is of interest because it represents an intermediate step in the GTPase reaction associated with ribosomal translocation during protein synthesis. A major uncertainty with regard to complex formation has been the requirement and function of the 30S ribosomal subunit. The present results agree with the assignment of the basic binding site of EF-G to the 50S ribosomal subunit; but more particularly, they demonstrate conclusively that the 30S subunit plays a role, serving to stabilize the complex once formed. Because of this, a requirement for the 30S subunit manifests itself whenever the experimental conditions fail to drive the reaction with 50S subunits in the direction of complex formation. This includes a low concentration of nucleotide or EF-G, and apparently also high concentrations of the monovalent cations K^+ and NH_4^+.

Qualitatively, the dependence of complex formation on the presence of the 30S subunit and the effect of an increasing concentration of monovalent cation or molar excess of EF-G over 50S subunits match similar observations on the GTPase reaction (Voigt et al., 1974; Parmeggiani et al., 1974). A similarity in the effect of NH_4^+ concentration on both complex formation and the GTPase reaction has also been observed by Arai and Kaziro (1975). However, the decline in GTPase activity of 50S plus 30S subunits when the monovalent cation concentration is lowered below a definite optimum is not paralleled by a decline in complex formation. Instead, complex formation with 50S plus 30S subunits remains fairly constant and nearly maximum. The ability of the 30S subunit to stabilize EF-G-ribosome interaction offers an explanation and gives some insight into regulation of the GTPase. At low monovalent cation concentrations, the 30S subunits likely stabilize the EF-G·GDP·ribosome complex to such an extent that its breakdown becomes severely rate limiting in the GTPase reaction. If true, saturation of the system should occur with a small excess of EF-G. And indeed, a 1.5 M excess of EF-G gives half-maximum activity in both the GTPase and complex formation at 2 and 10 mM NH_4^+, respectively. As the concentration of monovalent cation is raised, the 30S subunit may be less able to stabilize the complex so that GTPase activity increases until a point is reached where instability actually begins to interfere with GTP hydrolysis. The notion is supported by a decline in detectable complex just as GTPase activity peaks and starts to fall, and by the large increase in molar excess of EF-G needed for maximum GTPase activity (Parmeggiani et al., 1974). A picture emerges of the GTPase being controlled by the stability of the interaction between EF-G and the ribosome and with EF-G only temporarily binding the ribosome during the reaction. Any condition favoring complex formation too strongly will obviously be detrimental to the expression of a high rate of GTPase activity. Direct evidence for an exchange of EF-G between ribosomes during protein synthesis has been reported by Chinali and Parmeggiani (1973). The mutual exclusion of EF-G and elongation factor Tu for binding to the ribosome has also been interpreted in terms of EF-G exchange (for references, see Haselkorn and Rothman-Denes, 1973).

In the past, experiments concerning the ribosomal subunit requirements for complex formation with GDP and GTP have seemed conflicting. Bodley and Lin (1970) and Brot et al. (1971) reported that 50S subunits were fully capable of complex formation, whereas Parmeggiani and Gottschalk (1969), Hamel and Nakamoto (1972), and Sander et al. (1975) have reported a large stimulation by 30S subunits. It is now clear that these differences were due not only to the choice of NH_4^+ concentration, as suggested by Arai and Kaziro (1975), but also to the concentration of nucleotide and EF-G. Duration of the filtration procedure and the time required for centrifugation through sucrose gradients may also have played a role.

Only the ribosomal subunit requirement for GMPPCP binding demands further explanation. Brot et al. (1971), Acharya et al. (1973), and Arai and Kaziro (1975) have reported full complex formation with 50S subunits, while evidence both for and against a 30S subunit requirement has been presented from this laboratory (Parmeggiani and Gottschalk, 1969; Sander et al., 1972, 1975). In the present experiments, some preparations of 50S subunits showed complex forming ability with GDP, GTP, and GMPPCP. Others could form complex only with GDP and GTP unless the 30S subunit or 20% methanol was present. This discrimination between nucleotides suggests that GMPPCP binding may be somewhat more sensitive to 50S subunit conformation, which can vary from preparation to preparation, and offers an explanation for the observed inconsistencies.

Measurement of forward rate constants for complex formation with GDP shows that at low monovalent cations concentration accessibility of the EF-G binding site on the 50S subunit is changed little when the 30S subunit is added. These results do not support a mechanism of opening and closing of the binding site by the 30S subunit as suggested by Arai and Kariro (1975). Once EF-G and guanine nucleotide are bound, the presence of the 30S subunit strengthens the interaction considerably. This may be accomplished through direct contact of a part of EF-G with the 30S subunit, as already indicated by the results of Lee-Huang et al. (1974) and Marsh et al., (1975), or the presence of the 30S subunit may cause the complex to shift into a more stable conformation via interaction with the 30S subunit. Either way, a major function of the 30S subunit in the EF-G-ribosomal GTPase reaction appears to be the stabilization of the interaction between ribosomes and EF-G.

Acknowledgment

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References

Acharya, A. S., Moore, P. B., and Richards, F. M. (1973), *Biochemistry 12,* 3108-3114.

Arai, K., Kawakita, M., Kaziro, Y., Kondo, T., and Ui, N. (1973), *J. Biochem. (Tokyo) 73,* 1095-1105.

Arai, N., and Kaziro, Y. (1975), *J. Biochem. (Tokyo) 77,* 439-447.

Bodley, J. W., and Lin, L. (1970), *Nature (London) 227,* 60-61.

Bodley, J. W., Zieve, F. J., and Lin, L. (1970), *J. Biol. Chem. 45,* 5662-5667.

Bodley, J. W., Zieve, F. J., Lin, L., and Zieve, S. T. (1969), *Biochem. Biophys. Res. Commun. 37,* 437-443.

Brot, N., Spears, C., and Weissbach, H. (1969), *Biochem. Biophys. Res. Commun. 34,* 843-848.

Brot, N., Spears, C., and Weissbach, H. (1971), *Arch. Biochem. Biophys. 143,* 286-296.

Chinali, G., and Parmeggiani, A. (1973), *Eur. J. Biochem. 32,* 463-472.

Eckstein, F., Bruns, W., and Parmeggiani, A. (1975), *Biochemistry 14,* 5225-5232.

Hamel, E., and Nakamoto, T. (1972), *J. Biol. Chem. 247,* 6810-6816.

Haselkorn, R., and Rothman-Denes, L. B. (1973), *Annu. Rev. Biochem. 42,* 397-438.

Kuriki, Y., Inoue, N., and Kaziro, Y. (1970), *Biochim. Biophys. Acta 224,* 487-497.

Lee-Huang, S., Lee, H., and Ochoa, S. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 2928-2931.

Marsh, R. C., Chinali, G., and Parmeggiani, A. (1975), *J. Biol. Chem. 250,* 8344-8352.

Parmeggiani, A., and Gottschalk, E. M. (1969), *Biochem. Biophys. Res. Commun. 35,* 861-867.

Parmeggiani, A., Singer, C., and Gottschalk., E. M. (1971), *Methods Enzymol. 20,* 291-306.

Parmeggiani, A., Sander, G., Marsh, R. C., Voigt, J., Nagel, K., and Chinali, G. (1974), in Lipmann Symposium: En-

ergy, Regulation and Biosynthesis in Molecular Biology, Richter, D., Ed., Berlin, de Gruyter, pp 499-510.
Sander, G., Marsh, R. C., and Parmeggiani, A. (1972), *Biochem. Biophys. Res. Commun. 47,* 866-873.
Sander, G., Marsh, R. C., Voigt, J., and Parmeggiani, A. (1975), *Biochemistry 14,* 1805-1814.
Voigt, J., Sander, G., Nagel, K., and Parmeggiani, A. (1974), *Biochem. Biophys. Res. Commun. 57,* 1279-1286.

Mechanistic Interpretation of the Influence of Lipid Phase Transitions on Transport Functions†

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ABSTRACT: In an attempt to understand the mechanism by which a structural change of membrane lipids affects transport functions, the temperature dependence of transport rate has been measured to below the low temperature end of the fluid↔ordered phase transition of the membrane lipids. The unsaturated fatty acid requiring *Escherichia coli* strain T105 was supplemented with either *trans*-Δ^9-octadecenoate or *trans*-Δ^9-hexadecenoate or supplemented with and subsequently starved for *cis*-Δ^9-octadecenoate. Fluid↔ordered phase transitions measured in whole cells using the fluorescence probe *N*-phenyl-1-naphthylamine were compared with the temperature dependence of β-glucoside and β-galactoside transport. In addition to the previously observed downward "break" in the Arrhenius plot of transport rate which occurred near the middle of the phase transition temperature range, a second upward "break" was observed which could be correlated with the low-temperature end of the phase transition. These experiments are interpreted in terms of a partitioning of transport proteins between ordered and fluid domains which is described by a lateral distribution coefficient, κ. This distribution coefficient varies with the membrane lipid composition as well as with the transport system. Values for κ suggest a 2-20-fold preference for the partitioning of transport proteins into the fluid parts of the membrane.

Temperature-induced fluid↔ordered phase transitions of phospholipids in biological membranes are useful to study the structure-function relationships between membrane proteins and their lipid environment. The influence of a phase change on the catalytic action of a membrane embedded protein manifests itself as a change in reaction rate and/or a change in slope of the corresponding Arrhenius plot. Using unsaturated fatty acid requiring mutants of *Escherichia coli* (Silbert et al., 1974; Silbert, 1975), such changes in slope or "breaks" have been attributed to lipid phase transitions for a number of functions associated with the bacterial membrane (Schairer and Overath, 1969; Wilson et al., 1970; Overath et al., 1970; Esfahani et al., 1971; Overath and Träuble, 1973; Sackmann et al., 1973; Linden et al., 1973; Haest et al., 1974; Shechter et al., 1974).

Any correlation between changes in the reaction rate of a membrane function and a fluid↔ordered transition of the lipids depends on an unambiguous determination of the phase transition. Although a large number of methods are now available for the measurement of thermal transitions in membranes (cf. Melchior and Steim, 1976, for review), the most direct method remains the use of wide angle x-ray diffraction (Engelman, 1971; Shechter et al., 1974). The x-ray measurements show that within a certain temperature range domains of fluid and ordered lipids coexist and by comparison with suitable lipid standards they define the proportion of lipids taking part in the membrane transition. The high- and low-temperature boundaries of the transition range, T_h and T_l, are given by the temperatures where all the lipid molecules taking part in the transition have entered the fluid and ordered states, respectively. The width of the transition range, ΔT,[1] is given by $\Delta T = T_h - T_l$; the mid-transition temperature, T_t, can be defined as the temperature where one-half of the lipid is in either phase. Whereas the x-ray method defines the phase transition on a molecular basis, the convenient fluorescence technique using *N*-phenyl-1-naphthylamine (PhNap)[2] as a probe gives similar results regarding ΔT and T_t (Overath and Träuble, 1973; Overath et al., 1975). This method enables us to measure the lipid phase transition in whole cells under exactly the same conditions as the temperature dependence of membrane functions (Thilo and Overath, 1976).

Previous studies on the influence of a lipid phase transition on transport rate across the bacterial membrane revealed a biphasic shape for the Arrhenius plots (biphasic: cf. Figure 6a; Wilson et al., 1970; Overath et al., 1970; Esfahani et al., 1971; Overath and Träuble, 1973; Shechter et al., 1974). The rather limited number of experimental points led to extrapolations which suggested that, at a critical temperature, T_c, there is an abrupt change of activation energy, i.e., a "break" in the sense of a discontinuity. In the case of β-galactoside transport, the

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[1] This definition of ΔT differs from that used previously (cf. Overath and Träuble, 1973).

[2] Abbreviations used are: *cis*-Δ^9-18:1, *cis*-Δ^9-octadecenoic acid; *trans*-Δ^9-18:1, *trans*-Δ^9-octadecenoic acid; *trans*-Δ^9-16:1, *trans*-Δ^9-hexadecenoic acid; *cis,cis,cis*-$\Delta^{9,12,15}$-18:3, *cis,cis,cis*-$\Delta^{9,12,15}$-octadecatrienoic acid; PhNap, *N*-phenyl-1-naphthylamine; iPrSGal, isopropyl 1-thio-β-D-galactopyranoside; NphGal, *o*-nitrophenyl β-D-galactopyranoside; NphGlu, *p*-nitrophenyl β-D-glucopyranoside; CR buffer, Cohen-Rickenberg mineral salts medium.