Hotchkiss, R. D. (1944), *Advan. Enzymol. 4*, 153.
Kleinkauf, H., and Gevers, W. (1969), *Cold Spring Harbor Symp. Quant. Biol. 34*, 805.
Kleinkauf, H., Gevers, W., Roskoski, R., Jr., and Lipmann, F. (1970), *Biochem. Biophys. Res. Commun. 41*, 1218.
Kleinkauf, H., Roskoski, R., Jr., and Lipmann, F. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 2069.
Kurahashi, K., Yamada, M., Mori, K., Fujikawa, K., Kambe, M., Imae, Y., Sato, F., Takahashi, H., and Sakamoto, Y. (1969), *Cold Spring Harbor Symp. Quant. Biol. 34*, 815.
Lipmann, F. (1971), *Science 173*, 875.
Lipmann, F., Gevers, W., Kleinkauf, H., and Roskoski, R. Jr. (1971), *Advan. Enzymol. 35*, 1.
Ljones, T. O., Vaage, T. L., Zimmer, L. O., Frøholm, L. O., and Laland, S. G. (1968), *FEBS (Fed. Eur. Biochem. Soc. Lett. 1*, 339.
Mach, B., and Tatum, E. L. (1964), *Proc. Nat. Acad. Sci. U. S. 52*, 876.
Roskoski, R., Jr., Gevers, W., Kleinkauf, H., and Lipmann, F. (1970a), *Biochemistry 9*, 4839.
Roskoski, R., Jr., Kleinkauf, H., Gevers, W., and Lipmann, F. (1970b), *Biochemistry 9*, 4846.
Saito, Y., Otani, S., and Otani, S. (1970), *Advan. Enzymol. 33*, 337.
Sarges, R., and Witkop, B. (1965), *J. Amer. Chem. Soc. 87*, 2011.
Urry, D. W., Goodall, M. C., Glickson, J. D., and Mayers, D. F. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1907.

Effect of Polypeptide Chain Length on Dissociation of Ribosomal Complexes†

Robert J. Beller and Nicolette H. Lubsen*

ABSTRACT: The selectivity of Na^+ in distinguishing free ribosomes from those complexed with peptidyl-tRNA and mRNA (Beller, R. J., and Davis, B. D. (1971), *J. Mol. Biol. 55*, 477) suggested its usefulness in assessing the possible effect of the length of the nascent peptide on ribosome stability. Accordingly, ribosomes from *Escherichia coli* bearing peptides of defined length and composition were prepared *in vitro* with phage R17 RNA as messenger. Ribosomes bearing hot trichloroacetic acid precipitable nascent peptides were stable in Na^+ gradients, while the 70S initiation complex containing fMet-tRNA was completely dissociated under these conditions. However, gradients containing a high K^+ concentration showed that the completed initiation complex was more stable than free 70S ribosomes. Ribosomes complexed with tripeptidyl-tRNA (fMet-Ala-Ser-tRNA), prepared in the presence of fusidic acid, showed intermediate stability; when such ribosomes were analyzed in a Na^+ gradient the majority of the ribosomes was dissociated, but the tripeptide remained associated with the 50S subunit peak. Thus the degree of resistance to dissociation by salt, conferred on ribosomes by complexed peptidyl-tRNA, increases with increasing peptide length, and tripeptidyl-tRNA seems to have a greater affinity than fMet-tRNA for the peptidyl binding site on the 50S subunit. In contrast to the normal initiation complexes, those prepared with a nonhydrolyzable analog of GTP could not be distinguished in their stability from free ribosomes.

It is known that ribosomes complexed with mRNA and peptidyl-tRNA are not dissociated under various conditions that do cause dissociation of free ribosomes. These conditions include the addition of the ribosome dissociation factor (Subramanian *et al.*, 1969; Albrecht *et al.*, 1970), replacement of K^+ by Na^+ in sucrose gradient buffers (Beller and Davis, 1971), lowering of the Mg^{2+} concentration (Ron *et al.*, 1968; Oppenheim *et al.*, 1968; Kelly and Schaechter, 1969), elevation of the K^+ concentration (Edelman *et al.*, 1960; Martin *et al.*, 1969), and exposure of ribosomes to air in the absence of sulfhydryl compounds (Miyazawa and Tamaoki, 1967; Beller and Davis, 1970). This stabilization against dissociation might depend only on the presence of a bound tRNA, or it might also depend on the nature of the nascent peptide chain. Accordingly, we have prepared ribosomal complexes carrying nascent peptides of various defined lengths in extracts of *Escherichia coil*, with R17 phage RNA as messenger, and have compared them for their ability to survive gradient centrifugation under various ionic conditions. Furthermore, we have compared the stability of initiation complexes prepared with GTP or with the nonhydrolyzable analog, GMP·PCP.[1,2]

Materials and Methods

Preparation of Ribosomes and Factors. All subcellular components were prepared from *E. coli* strain MRE600 (Cammack and Wade, 1965), grown at 37° in minimal medium A (Davis and Mingioli, 1950), supplemented with 0.2% glucose and 0.2% Casamino Acids.

† From the Bacterial Physiology Unit, Harvard Medical School, Boston, Massachusetts 02115. *Received April 10, 1972.* This work was supported by grants to Dr. Bernard D. Davis from the National Institutes of Health and the American Cancer Society.

[1] A preliminary communication of this work appeared in *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 30*, 1311 Abs. (1971).

[2] Abbreviations used are: GMP·PCP, 5′-guanylylmethylenediphosphonate; IF, initiation factors; TKM, 10 mM Tris·HCl (pH 7.6)–50 mM KCl–5 mM magnesium acetate; TNaM, TKM with NaCl instead of KCl.

S30 and *S100* fractions were prepared according to the procedure described by Modolell and Davis (1968) except that 2 mM dithiothreitol was substituted for mercaptoethanol in the extraction buffer. S30 and S100 were stored in small portions at −70°.

To prepare *salt-washed ribosomes* the ribosomal pellet from the S100 preparation was dissolved in TNMD buffer (10 mM Tris·HCl (pH 7.6), 60 mM NH_4Cl, 10 mM magnesium acetate, and 1 mM dithiothreitol), one-third volume of 4 M NH_4Cl + 2 mM dithiothreitol was added, and the solution was allowed to stand overnight at 0°. The ribosomes were then pelleted by centrifugation for 2.5 hr at 150,000*g* and the supernatant fluid was removed; the pellet was resuspended in TNMD + 20% glycerol to an A_{260} of 200–500 and stored at 0° (salt-washed ribosomes).

To prepare *initiation factors* (IF) the supernatant from the salt-washed ribosomes was brought to 70% saturation by slow addition of $(NH_4)_2SO_4$. The resulting precipitate was collected by centrifugation at 9000*g* and was stored at −70°. Part of the pellet was freshly dissolved before use, at 10–20 mg of protein/ml in TNMD, and was dialyzed against the same buffer for 1–2 hr.

In Vitro Protein Synthesis. Polypeptide synthesis with phage R17 RNA as messenger was carried out according to the procedure of Modolell and Davis (1968). All final reaction mixtures contained: 50 mM Tris-HCl (pH 7.8), 60 mM NH_4Cl, 7.5 mM magnesium acetate, 10 mM reduced glutathione, 1 mM ATP–Tris, 0.02 mM GTP, 5 mM potassium phosphoenolpyruvate, and 30 μg of pyruvate kinase per ml. For synthesis of long peptides (hot trichloroacetic acid precipitable material), the following components were added: 0.03 mM [^{14}C]valine (66 Ci/mole), [^{12}C]amino acids minus valine at 0.05 mM, 1 mg of R17 RNA/ml, 0.3 volume of S30 extract, and 0.1 volume of S100. For short peptide synthesis the final reaction mixture contained 0.05 mM each of methionine and serine, 0.0125 mM [^{3}H]alanine (2 Ci/mmole), 1 mg of R17 mutant amB_2 (Gussin, 1966) RNA/ml, 0.15 volume each of preincubated S30 and S100, and 0.3 mg/ml of fusidic acid.

fMet-tRNA Binding to Ribosomes. Binding of fMet-tRNA to ribosomes was carried out according to a modification of the procedure of Modolell and Davis (1970). The reaction mixture contained: 50 mM Tris-HCl (pH 7.6), 100 mM NH_4Cl, 6.6 mM magnesium acetate, 6.6 mM 2-mercaptoethanol, 0.5 mM GTP (or GMP·PCP), 1.0 mg of IF/ml, 1.0 mg of R17 RNA/ml, 4×10^5 cpm of f[^{3}H]Met-tRNA/ml (3400 cpm/pmole), and 1.5 mg of salt-washed ribosomes/ml. Ribosomes were activated by heating for 2 min at 50° immediately before addition (Grunberg-Manago *et al.*, 1969).

Gradient Analysis. Aliquots of reaction mixture (40 or 100 μl) were layered on 4.8-ml 10–30% linear sucrose gradients in TKM (10 mM Tris·HCl (pH 7.6), 50 mM KCl, and 5 mM magnesium acetate) or TNaM (TKM with NaCl instead of KCl) and were centrifuged at 45,000 rpm in an SW50.1 rotor at 4° for 80 min. Gradients were analyzed with an Isco gradient fractionator with continuous monitoring at 254 nm. Ribosome distribution was measured by copying, cutting out, and weighing appropriate regions of the optical density tracings.

For determination of radioactivity in peptide-labeling experiments 5-drop fractions were collected, 1 drop of 0.5% bovine serum albumin was added, the protein was precipitated with 1 ml of 6% trichloroacetic acid + 1% Casamino Acids, and the precipitate was recovered on a Millipore filter. Where indicated in the text the samples were heated at 90° for 15 min before filtering. The filters were washed with 5% trichloroacetic acid, dried, and counted in toluene scintillation fluid.

Preparation of R17 RNA, f[^{3}H]Met-tRNA, and Formylated Standards for Electrophoresis. R17 RNA was prepared by the method of Gesteland and Boedtker (1964). The preparation of f[^{3}H]Met-tRNA (Modolell and Davis, 1970) was a gift from J. Modolell. The *N*-formylmethionine, fMet-Ala, and fMet-Ala-Ser used as standards for electrophoresis were prepared from commercially obtained unformylated compounds by the method of Sheehan and Yang (1958).

Paper Electrophoresis. The labeled peaks were collected from gradients and precipitated with trichloroacetic acid as described above. The precipitate was collected by centrifugation and redissolved in a small amount of 0.3 N NaOH, 5 μg of fMet-Ala, and 10 μg of fMet-Ala-Ser were added as markers, and the mixture was incubated at 0° for 15 min. With initiation complex preparations, containing f[^{3}H]Met label, the alkaline reaction mixture was neutralized with trichloroacetic acid and applied directly to the electrophoresis paper. With preparations containing short [^{3}H]Ala-labeled peptides the mixture was reprecipitated with trichloroacetic acid and centrifuged, and the resultant supernatant was applied to the electrophoresis paper. Paper electrophoresis was carried out with the system described by Kuechler and Rich (1970). Marker peptides were visualized by the platinic iodide method of Chargaff (1948). Strips of 1.0 or 0.5 cm were cut, suspended in 0.9 ml of H_2O, and counted in Bray's dioxane scintillation fluid.

Results

Stability of 70S Ribosomes Bearing Nascent Polypeptide Synthesized in Vitro. Ribosomes that are complexed with nascent polypeptide synthesized *in vivo* are stable when analyzed in TNaM gradients (Beller and Davis, 1971). The nascent peptides on such ribosomes should average half the length of the average completed polypeptide of *E. coli*. To determine the contribution of the length of the nascent peptide to the stability of the complexed ribosome, ribosomes bearing peptides of defined length and composition were prepared *in vitro* in cell-free extracts supplemented with the RNA of phage R17 as messenger. The major product synthesized is the coat protein of the phage (Nathans *et al.*, 1962; Nathans, 1965; Capecchi, 1966), whose amino acid sequence is known (Weber, 1967). In this system the ribosomes engaged in protein synthesis are found in monosomes and also, with the most active preparations, in short polysomes (Modolell and Davis, 1968).

Such a reaction mixture was allowed to incorporate amino acids, with radioactive valine, for several minutes; analysis in a TKM gradient (Figure 1a) showed that the ribosome-bound hot trichloroacetic acid precipitable nascent peptide was associated primarily with a large peak of 70S ribosomes. When analysis was performed instead in a TNaM gradient (Figure 1b) most of the 70S ribosomes were dissociated, but all of the radioactivity remained associated with the small residual peak of complexed 70S ribosomes. Since the first valine residue from the amino-terminal end in the coat protein of phage R17 occurs at position eight (Weber, 1967), these results show that ribosomes bearing nascent polypeptide at least eight amino acids long, and probably much longer on the average, are stable in a 50 mM Na^+ gradient, like the ribosomal complexes extracted from growing cells (Beller and Davis, 1971).

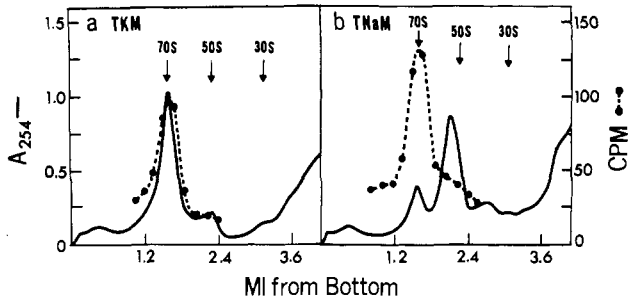

FIGURE 1: Stability in NaCl of ribosomes bearing hot trichloroacetic acid precipitable nascent peptide. After 15-min incubation at 37°, 40-μl portions of reaction mixture (see Methods) were layered on 4.8-ml 10–30% sucrose gradients in (a) TKM or (b) TNaM. Fractions collected directly from the gradient analyzer were precipitated, heated before being filtered, dried, and counted (see Methods). Arrows indicate the distances of sedimentation of 70S and subunit peaks in the TKM gradient.

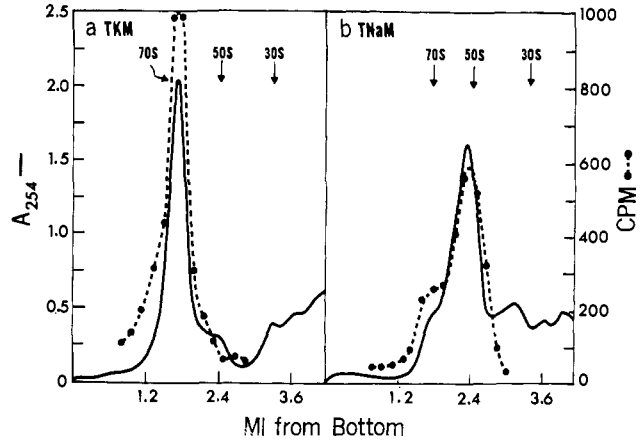

FIGURE 2: Stability in NaCl of ribosomes bearing peptide made in the presence of fusidic acid. The reaction mixture (see Methods) was incubated for 7 min at 37° and the reaction was stopped by chilling in an ice bath; 45-μl portions were layered on (a) TKM or (b) TNaM sucrose gradients. An additional 100-μl portion was layered on a second TNaM gradient for collection and analysis of bound nascent peptide (see Figure 3a,b). Gradients were centrifuged, and fractions were collected and cold trichloroacetic acid precipitable material was counted as in Figure 1. Arrows indicate the positions of 70S and subunit peaks in the TKM gradient.

Stability of 70S Ribosomes Bearing Tripeptidyl-tRNA. Ribosomes bearing a short peptide were prepared *in vitro* by incubation as above but in the presence of fusidic acid, which inhibits elongation factor G (Tanaka *et al.*, 1968). Figure 2 shows that on analysis in a TKM gradient peptide with labeled Ala (the second residue of the coat protein) was found associated with the 70S ribosome peak, but on analysis in a TNaM gradient most of the radioactivity was associated with the Na^+-derived 50S subunit peak, with a small amount remaining in the residual 70S peak.

The [^{3}H]Ala-labeled peptide associated with the 50S peak in a TNaM gradient was released from tRNA with alkali and was 90% acid-soluble. Electrophoretic analysis showed that this soluble material consisted entirely of fMet-Ala-Ser (Figure 3a), the amino-terminal tripeptide of the R17 coat protein. The poorly resolved residual 70S peak in the same gradient yielded only 54% acid-soluble material after base hydrolysis; and this material consisted primarily of the same tripeptide, but also contained longer peptides (Figure 3b), probably mostly pentapeptide, deduced from its electrophoretic mobility according to Keuchler and Rich (1970). (The tripeptide found in the 70S fraction may well represent contamination from the leading edge of the 50S peak.) A ribosome complexed with tripeptidyl-tRNA is thus less stable than ribosomes carrying long peptides, since the complex does not survive in TNaM gradients.

Though the mRNA used in these experiments contained an amber mutation in the coat protein gene, tripeptide was also the major product synthesized in the presence of fusidic acid when wild-type R17 RNA was used as messenger. Thus the nature of the mRNA did not influence the final product synthesized. The synthesis of tripeptide in the presence of fusidic acid, which is in disagreement with certain earlier published observations, will be discussed below.

Stability of the 70S Initiation Complex in Na^+. Bacterial protein synthesis is initiated with the pseudopeptide formylmethionine. When 70S initiation complexes[3] containing f[^{3}H]-Met-tRNA, prepared with salt-washed ribosomes and viral RNA, were analyzed in a TKM gradient a peak of radioactivity coincided with the 70S ribosomes (Figure 4a). This labeled material, when released from tRNA and analyzed electrophoretically, was found to consist entirely of f[^{3}H]-Met; no peptides of greater length were seen (Figure 5).

In a TNaM gradient this 70S peak did not survive and the radioactivity did not remain attached to any ribosomal particles (Figure 4b). Thus, the 70S initiation complex, like ribosomes complexed with tripeptidyl-tRNA, is less stable than ribosomes complexed with long nascent peptides. But fMet-

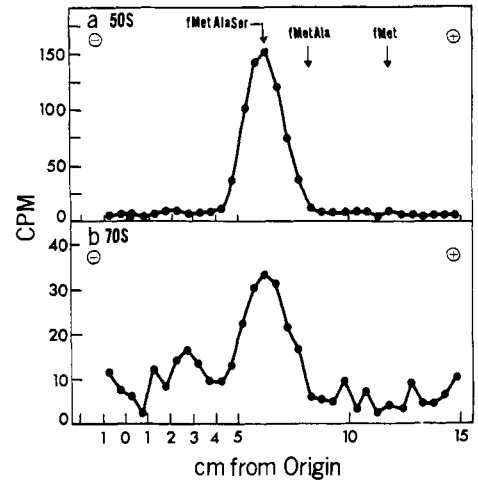

FIGURE 3: Electrophoretic analysis of ribosome-bound radioactive peptide synthesized with [^{3}H]alanine in the presence of fusidic acid. The 50S and 70S regions of a TNaM gradient from Figure 2 were collected and the bound nascent peptide was carried through two cycles of alkaline hydrolysis and acid precipitation as described in Methods; aliquots of all fractions were counted in order to determine the distribution of radioactivity between acid-soluble and acid-precipitable peptides. Radioactivity isolated from the 50S region was 89% acid soluble after base hydrolysis, and that from the 70S region was 54% acid soluble. Paper electrophoresis of original supernatant fractions was carried out, and electropherograms were analyzed, as described in Methods: (a) 50 S, (b) 70 S; note that alternate half-centimeter strips only were counted in part b. Arrows indicate the positions of marker peptides.

[3] For these experiments NH_4Cl-washed ribosomes were used. They are known to be sensitive to dissociation factor (Sabol *et al.*, 1970; Albrecht *et al.*, 1970; Subramanian and Davis, 1970) and thus may be considered free ribosomes.

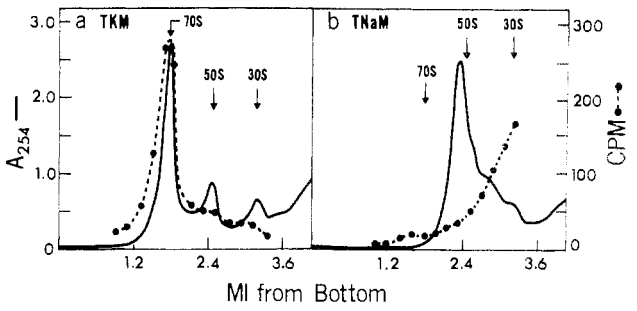

FIGURE 4: Stability in NaCl of 70S initiation complexes containing f[^{3}H]Met-tRNA. The reaction mixture (see Methods) was incubated for 7 min at 35°; 40-μl portions were layered on sucrose gradients in (a) TKM or (b) TNaM, and 100 μl was layered on a second TKM gradient for subsequent analysis of bound radioactive material (see Figure 5). Gradients were centrifuged, and fractions were collected and cold trichloroacetic acid precipitable material was counted, as in Figure 1.

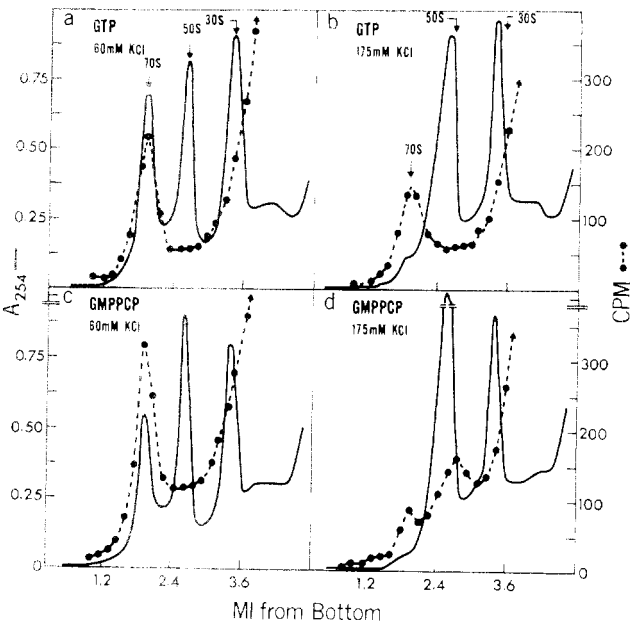

FIGURE 6: Stability in high KCl of 70S initiation complexes made in the presence of GTP or GMP·PCP. f[^{3}H]Met-tRNA binding was carried out as described in Methods except that one reaction mixture contained GMP·PCP (c,d) in place of GTP (a,b). 45-μl portions were layered on a second 60 mM KCl sucrose gradient for subsequent analysis of the bound radioactive material (see Figure 7). Gradients were analyzed as in Figure 4.

tRNA, in contrast to fMet-Ala-Ser-tRNA, evidently has lower affinity for the tRNA-binding site on the 50S subunit (compare with Figure 2b). The obvious trailing of radioactivity, and the displacement of the peaks to apparently larger s values (Figure 4b) suggest that dissociation of the initiation complex in a TNaM gradient was delayed until the ribosomes had passed some distance through the gradient and encountered the dissociating effect of high hydrostatic pressures (Infante and Baierlein, 1971; Spirin *et al.*, 1971; Hauge, 1971).

Stability of the 70S Initiation Complex in Elevated K$^+$. Since gradients containing 50 mM Na^+ failed to distinguish free ribosomes from those complexed with fMet-tRNA a more sensitive test for altered dissociability was sought. When salt-washed ribosomes were analyzed in gradients containing increasing concentrations of K^+, 175 mM KCl was found to be just sufficient to dissociate these free ribosomes into well-resolved subunit peaks under the centrifugal conditions used. At this concentration subunits form in the course of centrifugation and sediment somewhat ahead of 50S and 30S markers in the initial sample (Figure 6b,d), indicating that dissociation is delayed until the ribosomes have moved some distance through the gradient.

In a preparation containing 70S initiation complexes bearing labeled fMet-tRNA, as well as free ribosomes, the bound label sedimented with the 70S peak in a gradient containing 60 mM K^+ (Figure 6a). However, in a gradient containing 175 mM K^+ (Figure 6b) the majority of the 70S ribosomes were dissociated, but the labeled fMet-tRNA remained associated with the small residual 70S peak. Over 65% of the bound radioactivity was recovered in the 70S region of the 175 mM K^+ gradient in this experiment, and up to 85% in others. The peak of radioactivity was somewhat broader than in the 60 mM K^+ gradient, suggesting that dissociation may have begun far down in the gradient.

Stability of Initiation Complexes Prepared with GMP·PCP. Although the nonhydrolyzable methylene analog of GTP, GMP·PCP, promotes binding of fMet-tRNA to ribosomes, the fMet-tRNA bound does not react with puromycin, in contrast to that bound in the presence of GTP (Hershey and Thach, 1967). It was thus of interest to determine whether the hydrolysis of GTP during the formation of the initiation complex increased the stability of this complex. It was found that 70S initiation complexes prepared with GMP·PCP were not stable to centrifugation through a sucrose gradient containing 175 mM KCl (Figure 6d); only 15% of the label sedimented in the 70S region. Figure 7 presents evidence that the 70S initiation complex made in the presence of GMP·PCP, and isolated in TKM, indeed contained label only in fMet-tRNA, like the complex made with GTP (Figure 4).

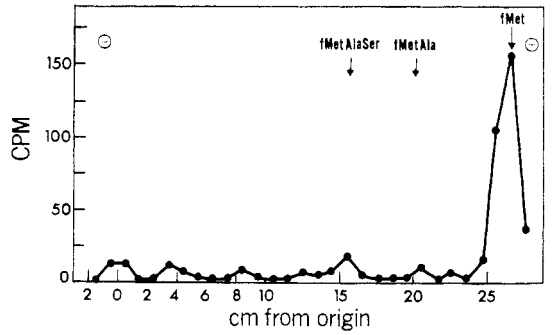

FIGURE 5: Electrophoretic analysis of ribosome-bound radioactive material in 70S complexes with f[^{3}H]Met-tRNA made in the presence of GTP. The 70S region from a TKM gradient from the experiment of Figure 4 was collected and treated as described in Methods. Electrophoresis was as in Figure 3, and the electropherogram was cut into centimeter strips and counted.

Discussion

Influence of Polypeptide on Dissociation. We have shown earlier (Beller and Davis, 1971) that free ribosomes dissociate during centrifugation in sucrose gradients containing NaCl (TNaM) instead of the usual KCl, while complexed ribosomes are stable under these conditions. This result suggested that alteration in monovalent ions might be useful in assessing the contribution of nascent peptides of different lengths to ribo-

some stability. Accordingly, complexed 70S ribosomes bearing polypeptidyl-tRNA, tripeptidyl-tRNA, or fMet-tRNA were prepared *in vitro* with R17 RNA as messenger.

Ribosomes complexed with long polypeptides were stable in gradients containing TNaM (Figure 1). Complexes containing labeled tripeptidyl-tRNA (fMet-Ala-Ser-tRNA) were less stable. In a TNaM gradient they were dissociated, but most of the tripeptide, unlike labeled fMet-tRNA, remained associated with the large subunits (Figure 2). (This result supports the early suggestion that peptidyl-tRNA remains bound to the 50S subunit under borderline conditions causing dissociation: Gilbert, 1963; Schlessinger and Gros, 1963; Leder *et al.*, 1969.) Initiation complexes containing fMet-tRNA were even less stable, being completely dissociated and freeing the fMet-tRNA in TNaM gradients (Figure 4). However, these complexes are somewhat more stable than free ribosomes, for only the latter dissociated in gradients containing 175 mM KCl (Figure 6).

These results show that the degree of resistance of complexed 70S ribosomes to dissociation by monovalent cations during gradient centrifugation depends on the length of the nascent peptidyl-tRNA. Moreover, it appears that tripeptidyl-tRNA is bound more tightly than fMet-tRNA to the tRNA binding site on the large subunit. Though these results do not rule out the possibility that ribosome stability is also influenced by composition of the nascent peptide or by the position of the ribosome on the messenger molecule, the importance of polypeptide length has been indicated by work of Jost *et al.* (1968), with a system that was less physiological but permitted variation in length without variation in composition. When they formed complexed 70S ribosomes from subunits in the presence of poly(A) and oligolysyl-tRNA the affinity for the latter appeared to increase with chain length, in the range from two to six amino acids.

We have also observed that the initiation complex formed with GTP is significantly more stable than that formed with GMP·PCP (Figure 6). Since Thach and Thach (1971) have shown that no translocation occurs during fMet binding with GTP, the added stability must result from a "locking" of fMet-tRNA into the donor (puromycin-reactive) site.

An intimate association between the ribosome and the tRNA-proximal portion of the growing peptide chain is also suggested by the observation that on mammalian ribosomes 30–40 amino acids are protected from digestion with proteolytic enzymes (Malkin and Rich, 1967; Blobel and Sabatini, 1970). Moreover, Panet *et al.* (1970) have shown that both chain length and composition may influence the reactivity of nascent oligopeptide with puromycin.

Action of Fusidic Acid. We found that when an *in vitro* protein-synthesizing system was inhibited with fusidic acid the major product synthesized was the amino-terminal tripeptide of the phage coat protein, fMet-Ala-Ser (Figure 3). Others have found that in the presence of fusidic acid, with natural or synthetic messengers, only dipeptide was synthesized (Leder *et al.*, 1969; Roufa *et al.*, 1970). However, these experiments were carried out in purified systems with ribosomes depleted of G factor. We used an S30 extract supplemented with an equal volume of S100 (Figure 2); and from an estimate (Gordon, 1970) of the amount and distribution of elongation factor G in cell extracts our reaction mixture would be expected to contain about 1.5 molecules of this factor per ribosome. Bodley *et al.* (1969), as well as other groups (Kuriki *et al.*, 1970; Okura *et al.*, 1970), have shown that in the presence of fusidic acid one round of G-factor-mediated GTP hydrolysis (and, therefore, presumably also of translocation) can indeed take place: the antibotic prevents dissociation of the resulting ribosome·G factor·GDP complex. Thus, in a G-factor-depleted system with fusidic acid a few ribosomes should tie up the small amount of factor, preventing it from cycling onto additional ribosomes, and so most ribosomes would fail to undergo translocation, while a molar ratio of G factor to ribosomes exceeding 1.0 would permit one translocation per ribosome. This model is consistent with the demonstration by Kaji *et al.* (1969) that the inhibitory effect of fusidic acid on the G-factor-dependent tRNA release reaction could be overcome by excess G factor.

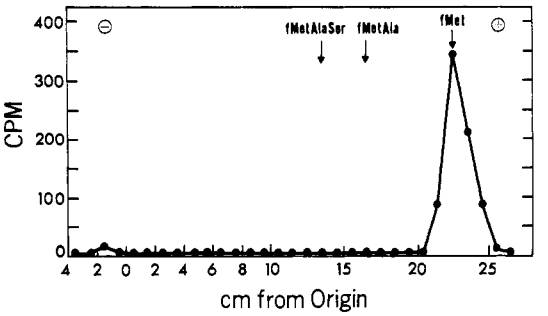

FIGURE 7: Electrophoretic analysis of radioactive material in 70S complexes made in the presence of GMP·PCP. The 70S region from a 60 mM KCl sucrose gradient from the experiment of Figure 6 was collected and treated as described in Methods. The radioactive nascent peptide was analyzed electrophoretically as in Figure 5.

While our finding of tripeptide synthesis in the presence of fusidic acid and a molar excess of G factor is thus consistent with a specific action of this antibiotic on translocation, it is not so readily reconciled with recent evidence that fusidic acid also impairs access of aminoacyl-tRNA to the A site (Cabrer *et al.*, 1972; Miller, 1972; Richman and Bodley, 1972). The question whether fusidic acid inhibits the ribosome primarily at translocation or at the binding of aminoacyl-tRNA is under investigation.

Acknowledgment

The authors express their thanks to Dr. Bernard D. Davis for his encouragement and many valuable comments during the course of this work and preparation of the manuscript.

References

Albrecht, J., Stap, F., Voorma, H. O., Van Knippenberg, P. H., and Bosch, L. (1970), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 6*, 297.

Beller, R. J., and Davis, B. D. (1970), *Bacteriol. Proc., Amer. Soc. Microbiol.*, 49.

Beller, R. J., and Davis, B. D. (1971), *J. Mol. Biol. 55*, 477.

Blobel, G., and Sabatini, D. D. (1970), *J. Cell Biol. 45*, 130.

Bodley, J. W., Zieve, F. J., Lin, L., and Zieve, S. T. (1969), *Biochem. Biophys. Res. Commun. 42*, 475.

Cabrer, B., Vazquez, D., and Modolell, J. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 733.

Cammack, K. A., and Wade, H. E. (1965), *Biochem. J. 96*, 671.

Capecchi, M. R. (1966), *J. Mol. Biol. 21*, 173.

Chargaff, E. (1948), *J. Biol. Chem. 175*, 67.

Davis, B. D., and Mingioli, E. S. (1950) *J. Bacteriol. 60*, 17.

Edelman, I. S., Ts'o, P. O. P., and Vinograd, J. (1960), *Biochim. Biophys. Acta 43*, 393.

Gesteland, R. F., and Boedtker, H. (1964), *J. Mol. Biol. 8*, 496.

Gilbert, W. (1963), *J. Mol. Biol. 6*, 389.

Gordon, J. (1970), *Biochemistry 9*, 912.
Grunberg-Manago, M., Clark, B. F. C., Revel, M., Rudland, P. S., and Dondon, J. (1969), *J. Mol. Biol. 40*, 33.
Gussin, G. M. (1966), *J. Mol. Biol. 21*, 435.
Hauge, J. G. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 17*, 168.
Hershey, J. W. B., and Thach, R. E. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 759.
Infante, A. A., and Baierlein, R. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1780.
Jost, M., Shoemaker, N., and Noll, H. (1968), *Nature (London) 218*, 1217.
Kaji, A., Igarishi, K., and Ishitsuka, H. (1969), *Cold Spring Harbor Symp. Quant. Biol. 34*, 167.
Kelley, W. S., and Schaechter, M. (1969), *J. Mol. Biol. 42*, 599.
Kuechler, E., and Rich, A. (1970), *Nature (London) 225*, 920.
Kuriki, Y., Inoue, N., and Kaziro, Y. (1970), *Biochim. Biophys. Acta 224*, 487.
Leder, P., Bernardi, A., Livingston, D., Loyd, B., Roufa, D., and Skogerson, L. (1969), *Cold Spring Harbor Symp. Quant. Biol. 34*, 411.
Malkin, L. I., and Rich, A. (1967), *J. Mol. Biol. 26*, 329.
Martin, T. E., Rolleston, T. S., Low, R. B., and Wool, I. G. (1969), *J. Mol. Biol. 43*, 135.
Miller, D. L. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 752.
Miyazawa, F., and Tamaoki, T. (1967), *Biochim. Biophys. Acta 134*, 470.
Modolell, J., and Davis, B. D. (1968), *Proc. Nat. Acad. Sci. U. S. 61*, 1279.
Modolell, J., and Davis, B. D. (1970), *Proc. Nat. Acad. Sci. U. S. 67*, 1148.
Nathans, D. (1965), *J. Mol. Biol. 13*, 521.
Nathans, D., Notani, G., Schwartz, J. H., and Zinder, N. D. (1962), *Proc. Nat. Acad. Sci. U. S. 48*, 1424.
Okura, A., Kinoshita, T., and Tanaka, N. (1970), *Biochem. Biophys. Res. Commun. 41*, 1545.
Oppenheim, J., Sheinbuks, J., Biava, C., and Marcus, L. (1968), *Biochim. Biophys. Acta 161*, 386.
Panet, A., de Groot, N., and Lapidot, Y. (1970), *Eur. J. Biochem. 15*, 222.
Richman, N., and Bodley, J. W. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 686.
Ron, E. Z., Kohler, R. E., and Davis, B. D. (1968), *J. Mol. Biol. 36*, 83.
Roufa, D. J., Skogerson, L. E., and Leder, P. (1970), *Nature (London) 227*, 567.
Sabol, S., Sillero, M. A. G., Iwasaki, K., and Ochoa, S. (1970), *Nature (London) 228*, 1269.
Schlessinger, D., and Gros, F. (1963), *J. Mol. Biol. 7*, 350.
Sheehan, J. C., and Yang, D.-D. H. (1958), *J. Amer. Chem. Soc. 80*, 1154.
Spirin, A. S., Sabo, B., and Kovalenko, V. A. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 15*, 197.
Subramanian, A. R., and Davis, B. D. (1970), *Nature (London) 228*, 1273.
Subramanian, A. R., Davis, B. D., and Beller, R. J. (1969), *Cold Spring Harbor Symp. Quant. Biol. 34*, 223.
Tanaka, N., Kinoshita, T., and Masukawa, H. (1968), *Biochem. Biophys. Res. Commun. 30*, 278.
Thach, S. S., and Thach, R. E. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1791.
Weber, K. (1967), *Biochemistry 6*, 3144.

Oxygenation of Polyunsaturated Fatty Acids during Prostaglandin Biosynthesis by Sheep Vesicular Gland†

William L. Smith and William E. M. Lands*

ABSTRACT: We found that two types of fatty acid dioxygenase activity are present in acetone powder preparations of sheep vesicular gland. One activity (E_a) when stimulated by phenol may be suppressed by the functioning of glutathione peroxidase. Therefore, E_a seems to require hydroperoxide as an obligatory intermediate. The oxygenation of fatty acids catalyzed by freshly prepared homogenates of sheep vesicular gland was also inhibited by glutathione peroxidase in the presence of glutathione. The slower oxygenase activity (E_b) found in untreated vesicular gland acetone powder preparation was not affected by glutathione peroxidase and therefore did not seem to require hydroperoxide in its mechanism of action. E_b activity was inactivated in the presence of both substrates, fatty acid and oxygen, by a process which appeared to be first order with respect of enzyme concentration. E_a was inactivated by a kinetically similar process, and in addition also lost activity at a significant rate in the presence of hydroperoxide alone, apparently by a substrate-independent process. The kinetic formulation of E_a action resembles that for soybean lipoxygenase in being product activated and self-destructive.

The oxidative cyclization of certain polyunsaturated acids to form prostaglandins involves a series of reactions (Bergström, 1967; Samuelsson, 1967, 1969; Nugteren *et al.*, 1966). Two principal features indicated by the work of Samuelsson and coworkers are the initial attack by a dioxygenase (Samuelsson, 1965; Hamberg and Samuelsson, 1967a) and the existence of a cyclic endoperoxide intermediate (Samuel-

† From the Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48104. *Received March 6, 1972.* This work was supported in part by a grant (GB-8243) from the National Science Foundation and a U. S. Public Health Service Health Science Predoctoral Traineeship (W. L. S.).