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Reconstitution of Qβ RNA replicase from a covalently bonded elongation factor Tu·Ts complex

(RNA phage/RNA-dependent RNA nucleotidyltransferase/renaturation/crosslinking/dimethyl suberimidate)

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ABSTRACT *Escherichia coli* phage Qβ RNA replicase, an RNA-dependent RNA polymerase (RNA-dependent RNA nucleotidyltransferase), is a tetramer composed of one phage-coded polypeptide and three host-supplied polypeptides which are known to function in the biosynthesis of proteins in the uninfected host. Two of these polypeptides, protein synthesis elongation factors EF-Tu and EF-Ts, can be covalently crosslinked with dimethyl suberimidate to form a complex which lacks the ability to catalyze the known host functions catalyzed by the individual elongation factors. Using a previously developed reconstitution system we have examined the effects of crosslinking the EF-Tu·Ts complex on reconstituted replicase activity. Renaturation is significantly more efficient when exogenously added native EF-Tu·Ts is crosslinked than when it is not. Crosslinked EF-Tu·Ts can be purified from a crude crosslinked postribosomal supernatant by its ability to replace EF-Tu and EF-Ts in the renaturation of denatured Qβ replicase. A sample of Qβ replicase with crosslinked EF-Tu·Ts replacing the individual elongation factors was prepared. Although it lacked EF-Tu and EF-Ts activities, it could initiate transcription of both poly(C) and Qβ RNA normally and had approximately the same specific activity as control enzyme. Denatured Qβ replicase formed with crosslinked EF-Tu·Ts was found to renature much more rapidly than untreated enzyme and, in contrast to normal replicase, its renaturation was not inhibited by GDP. The results demonstrate that EF-Tu and EF-Ts function as a complex in Qβ replicase and do not perform their known protein biosynthetic functions in the RNA synthetic reaction.

Qβ replicase, the RNA polymerizing enzyme induced in *Escherichia coli* after infection by the single-stranded RNA bacteriophage, Qβ, is composed of four nonidentical subunits: one phage-coded polypeptide and three host-coded subunits (1, 2). The smaller two host polypeptides are the protein biosynthesis elongation factors EF-Tu and EF-Ts (3), which are involved in the binding of aminoacyl-tRNA to ribosomes (4). The role played by the protein synthesis elongation factors in the RNA-dependent RNA synthetic reaction is not known, but two hypotheses deriving from the known mechanism of EF-Tu action have been advanced (3, 5): first, EF-Tu contains a GTP-binding site (4), and GTP is the only nucleoside triphosphate with which Qβ replicase can initiate transcription (6). Thus EF-Tu could supply the initiating GTP and EF-Ts could then function to remove the enzyme from the 5′ end of the nascent chain, in a manner analogous to its action in protein biosynthesis (4). Second, the aminoacyl-tRNA binding site of EF-Tu might also be involved in initiation of transcription by Qβ replicase, since both aminoacyl-tRNA and Qβ RNA end in CCA at their 3′ terminus (7). The finding that EF-Tu and EF-Ts were necessary for initiation of transcription but not elongation with synthetic templates (5) was consistent with either of these hypotheses. The observation that inactivation of the aminoacyl-tRNA binding site of EF-Tu by sulfhydryl reagents without a concomitant inhibition of Qβ replicase activity with either poly(C) or Qβ RNA (5, †) as template eliminated the possibility of involvement of that site in the RNA synthetic reaction.

Using a recently developed reconstitution system (8), we have obtained evidence that EF-Tu and EF-Ts may not perform functions in Qβ replicase analogous to their known functions in protein biosynthesis: reagents that prevent formation of the EF-Tu·Ts complex, GDP and kirromycin, are potent inhibitors of renaturation of denatured Qβ replicase†. This suggested that the EF-Tu·Ts complex, rather than the individual polypeptides, is functional in the RNA synthetic reaction. Other than its role as an intermediate in the regeneration of EF-Tu·GTP from EF-Tu·GDP, the EF-Tu·Ts complex has no known host function. In the experiments reported here we have prepared a sample of Qβ replicase in which the native EF-Tu and EF-Ts are replaced by an EF-Tu·Ts complex covalently bonded together by treatment with dimethyl suberimidate, a bifunctional protein crosslinking reagent. Since the enzyme (hereinafter referred to as "crosslinked" replicase) is capable of apparently normal synthesis with all templates and since the GTP/GDP binding and exchange activities associated with the protein synthesis elongation factors have been inactivated, the results demonstrate that these activities are not required for the EF-Tu·Ts to function in the RNA synthetic reaction, and that most likely this reaction requires the EF-Tu·Ts complex.

RESULTS

Crosslinked EF-Tu·Ts functions in renaturation

When Qβ replicase, denatured in 8 M urea solution, is renatured, its rate of renaturation is limited by the renaturation of EF-Tu (8). If the denatured replicase is renatured by dilution with buffer containing native EF-Tu·GDP or EF-Tu·Ts, the rate of recovery of RNA synthetic activities is greatly stimulated (8). At short times after initiation of renaturation the Qβ replicase recovered is formed from the exogenous native elongation factors, since the endogenous EF-Tu has not yet renatured. In the experiment shown in Fig. 1, denatured replicase was renatured in the presence of increasing concentrations of undenatured EF-Tu·Ts and the percent of original poly(C)-dependent poly(G) polymerase activity recovered after 5 min at 2° was measured. The rate of renaturation is directly dependent on the concentration of EF-Tu·Ts added. Treatment of EF-Tu·Ts with dimethyl suberimidate, a protein crosslinking reagent, results in inactivation

Abbreviations: EF-Tu and EF-Ts, protein biosynthesis elongation factors Tu and Ts, respectively; EF-Tu·Ts, the complex of EF-Tu and EF-Ts.

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† S. Brown and T. Blumenthal, *J. Biol. Chem*, in press.

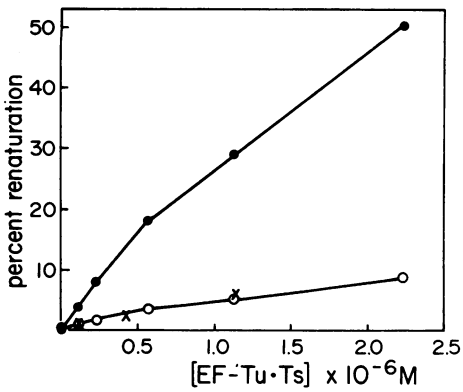

FIG. 1. Renaturation of Qβ replicase stimulated by EF-Tu·Ts and crosslinked EF-Tu·Ts. Qβ replicase purified by the method of Kamen (11) was denatured in 8 M urea (final concentration in renaturation buffer = 0.36 μM), incubated at 21° for 2 hr, and renatured in high-salt buffer containing EF-Tu·Ts (○) or crosslinked EF-Tu·Ts (●) at the concentration shown, as described (8). EF-Tu·Ts was made from purified elongation factors (12, 13). A sample was dialyzed into triethanolamine buffer and crosslinked with dimethyl suberimidate for 1 hr at 10°, as described (14). The reaction was quenched with a 5-fold molar excess of NH_4Cl before addition of elongation factors to renaturation buffer. EF-Tu and EF-Ts were also treated individually with crosslinker as above and added to the renaturation buffer at the three concentrations shown (×). The control EF-Tu·Ts was treated with already-quenched dimethyl suberimidate at the same final concentration. Renaturation was allowed to proceed for 5 min at 2° before samples were removed and assayed for poly(G) polymerase activity as described (8). Percent renaturation was calculated from control tubes in which nondenatured enzyme was diluted to the same concentration in the same buffer containing the same concentrations of crosslinked and control elongation factors, incubated at 2°, and assayed at 5 min.

of more than 90% of the GDP binding (EF-Tu) and exchange (EF-Ts) activities (unpublished observations and Table 1). To our surprise, the crosslinked EF-Tu·Ts not only retained its ability to function in the renaturation of the enzyme, it was significantly more effective than untreated EF-Tu·Ts complex in stimulating the rate of recovery of activity (Fig. 1). In a control experiment the two elongation factors were treated separately with the crosslinker and the reaction was quenched before the subunits were mixed together so that they were not covalently linked. In this case the EF-Tu GTP binding and EF-Ts exchange activities were not lost and the rate of recovery of Qβ replicase activity was not stimulated over that provided by untreated EF-Tu·Ts (Fig. 1, ×). Although the stimulation of renaturation by EF-Tu·Ts is prevented by GDP†, which dissociates the EF-Tu·Ts complex (4), the stimulation by crosslinked EF-Tu·Ts is not (data not shown, but see Fig. 4). Thus, the crosslinked EF-Tu·Ts must be the active moiety in the renaturation of the enzyme.

Purification of crosslinked EF-Tu·Ts

In order to obtain additional evidence that the crosslinked EF-Tu·Ts was active in Qβ replicase, we crosslinked a crude postribosomal supernatant with dimethyl suberimidate and purified for the activity that stimulated the renaturation of denatured Qβ replicase at 2°. GDP and kirromycin were added to the renaturation mixture to prevent uncrosslinked EF-Tu·Ts from stimulating renaturation†. The stimulating protein was purified by $(NH_4)_2SO_4$ precipitation, DEAE-Sephadex chromatography, heat treatment, passage through phosphocellulose, and gel filtration. It was found to elute

Table 1. Specific activities of native and "crosslinked" Qβ replicase

	Native replicase	"Crosslinked" replicase
Specific activity* [poly(C)], units/mg	9,000	11,100
Initiation activity†, (pmol of chains initiated/ pmol of protein)		
Qβ RNA	0.17	0.29
poly(C)	0.62	0.76
EF-Tu activity‡, (pmol of GDP bound/ pmol of protein)	0.86	0.07
EF-Ts activity‡, (pmol of EF-Ts activity/ pmol of protein)	0.57	0.03

* Protein concentration was determined by measurement of absorbance (A_{280}). $E^{1\ mg/ml}_{280\ nm}$ = 1.5 and a molecular weight of 215,000 were used to determine molarity. Specific activity is nmol of GMP incorporated in 10 min at 37° in the standard poly(C)-dependent poly(G) polymerase assay (11).

† Initiations on Qβ RNA were quantitated by initiating 0.23 pmol of replicase with varying amounts of Qβ RNA plus saturating host factor [S. Brown and T. Blumenthal (1976) *J. Biol. Chem.* in press] in the presence of 1 mM each GTP and ATP at 36° for 5 min under conditions described by Kamen (11). Then 10 μM aurintricarboxylic acid was added, followed by the remaining NTPs with [^{3}H]UTP (77 cpm/pmol), and the mixture was incubated at 36° for 5 min. Samples were precipitated and filtered as described by Kamen (11). No RNA backgrounds (0.9 pmol of UMP) were subtracted, and the number of (−) strands completed was determined by assuming 1034 pmol of UMP/pmol of (−) strands, using values reviewed by Boedker and Gestland (17). Initiations on poly(C) were quantitated in the standard poly(G) polymerase assay (11) with aurintricarboxylic acid added after 30 sec to prevent reinitiation (9). Poly(C), purchased from P.L. Biochemicals (Milwaukee), was approximately 10 S, so a molecular weight of 500,000 was used for calculation of the number of chains synthesized.

‡ EF-Tu GDP-binding activity and EF-Ts exchange activity were measured as described (8).

from Sephadex G-100 equilibrated with 20 μM GDP with an average partition coefficient (K_{av}) of 0.05 and migrated in sodium dodecyl sulfate polyacrylamide gels with an approximate molecular weight of 80,000 (Fig. 2, gel 1). The GDP binding activity eluted from Sephadex G-100 with a K_{av} of 0.13 under these conditions. The G-100 fractions containing the stimulatory activity did not bind detectable amounts of GDP, although they did crossreact with antibodies prepared against EF-Tu and EF-Ts (not shown). Thus, cross-linked EF-Tu·Ts can be purified from a mixture of crosslinked proteins by virtue of its ability to replace EF-Tu and EF-Ts during the renaturation of denatured Qβ replicase.

Preparation of "crosslinked" replicase

In order to determine whether crosslinked EF-Tu·Ts could replace entirely the individual elongation factors in Qβ replicase, we used our denaturation-renaturation system to prepare a sample of enzyme without EF-Tu and EF-Ts activities. A sample of pure native Qβ replicase was denatured by the addition of crystalline urea to 8 M and the urea diluted out with high-salt buffer containing 100 μM GDP (to prevent endogenous uncrosslinked EF-Tu·Ts from forming) and the partially purified stimulatory protein. The mixture was incubated at 2° for 15 min to allow the replicase to re-

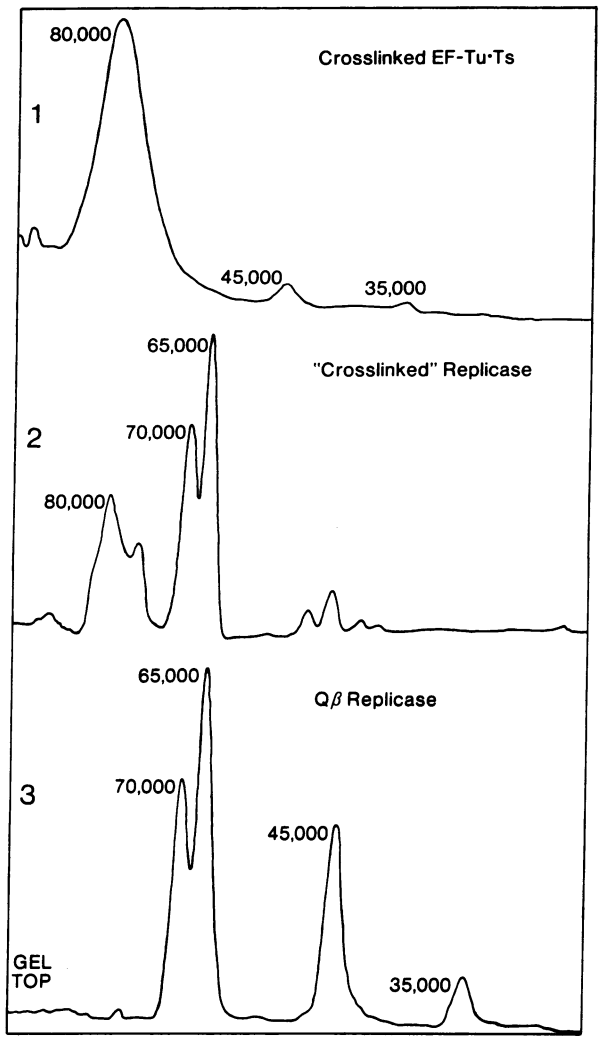

FIG. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of crosslinked EF-Tu·Ts, "crosslinked" Qβ replicase, and Qβ replicase. Crosslinked EF-Tu·Ts was prepared from *E. coli* MRE600 as follows: The presence of the crosslinked elongation factors was assayed by stimulation of renaturation of denatured Qβ replicase in the presence of 0.2 mM kirromycin and 0.1 mM GDP after 15 min at 2° as described (8). The renatured samples were assayed for poly(G) polymerase activity without pyruvate kinase and phospho*enol*pyruvate. Cells (150 g) were broken and a postribosomal supernatant was prepared and concentrated by addition of $(NH_4)_2SO_4$ to 80% saturation as described by Arai *et al.* (12). The resuspended precipitate was dialyzed against triethanolamine buffer pH 8.0 (14), centrifuged at 27,000 × *g* for 20 min at 4°, and passed through a 2.5 × 45 cm Sephadex G-25 column. The excluded material was crosslinked at a final concentration of 0.7 mg/ml with dimethyl suberimidate (5 mg/ml) for 20 min at 21°. The pH of the solution was raised to 10.0 with liquid triethanolamine and the solution was incubated 5 min before the pH was returned to 8.0 with 50% (vol/vol) glacial acetic acid. The rise to pH 10 stabilizes the crosslinked product (15). The solution was then cooled to 4° and concentrated with $(NH_4)_2SO_4$ as before. The resuspended precipitate was dialyzed against standard buffer [20 mM Tris·HCl pH 7.5, 10 mM $Mg(OAc)_2$, 10% glycerol, 50 mM KCl, and 0.5 mM dithioerythritol] and chromatographed on a 2.5 × 43 cm DEAE-Sephadex A-50 column. It was eluted with a 1.6-liter 0.05 to 0.25 M KCl gradient in standard buffer and assayed for stimulation of Qβ replicase renaturation as described above. The stimulating fractions (eluting at 0.18 M KCl) were concentrated by $(NH_4)_2SO_4$ precipitation, resuspended, and dialyzed as before. The material was heated to 55°, incubated 5 min, cooled, and clarified by centrifugation at 25,000 × *g* for 15 min at 4°. The remaining material was dialyzed into standard buffer with 30 mM $(NH_4)_2SO_4$ and no $Mg(OAc)_2$ and passed through a 2-ml phosphocellulose column. It was then concentrated as before, resuspended, dialyzed against standard buffer with 20 μM GDP, and chromatographed on a 2.5 × 160 cm Sephadex G-100 column. Fractions stimulating renaturation were pooled and dialyzed against renaturation buffer (8). The final volume was 25 ml with an $A_{280} = 0.3$. Gel 1, 20 μl of the dialyzed G-100 pool.

One milliliter of pure Qβ replicase (1.7 mg/ml) was denatured by the addition of crystalline urea to 8 M, incubated 2 hr at 21°, and then diluted with 10 ml of the dialyzed G-100 pool with 100 μM GDP. After a 15-min incubation at 2°, the solution was further diluted with 100 ml of standard buffer, 20% glycerol, without $Mg(OAc)_2$ and loaded onto a 2-ml phosphocellulose column. The column was washed with 5 ml of the above buffer plus 50 mM NaCl and eluted with 0.4 M NaCl-containing buffer. Approximately 0.2 mg of protein in 1 ml was recovered. Gel 2, 10 μl of the phosphocellulose eluate ("crosslinked" Qβ replicase, 0.23 mg/ml). Gel 3, 10 μl of Qβ replicase (0.30 mg/ml). The proteins were electrophoresed on 10% polyacrylamide sodium dodecyl sulfate gels as described by Weber and Osborn (16), and scanned at A_{569}.

nature in the presence of the crosslinked preparation. Renaturation was then stopped by diluting out the salt, and the renatured enzyme was loaded onto phosphocellulose. The bound material was eluted with buffer containing 0.4 M NaCl. This high-salt eluted material was found to contain Qβ replicase of high specific activity (Table 1) and capable of crossreaction with antibodies prepared against EF-Tu and EF-Ts (not shown). Very low amounts of GDP binding and GDP exchange activities were found to be present (Table 1). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (Fig. 2, gel 2) showed a virtual absence of bands corresponding to EF-Tu and EF-Ts but the presence of a prominent band corresponding to a molecular weight of about 80,000, which is the expected weight of EF-Tu·Ts. Since antigenicity with anti-EF-Tu and anti-EF-Ts was present, while the EF-Tu and EF-Ts appeared to be absent by both physical and functional criteria, we conclude that crosslinked EF-Tu·Ts complex has replaced the individual elongation factors.

Properties of "crosslinked" replicase

We present in Table 1 some properties of the "crosslinked" replicase compared with those of control enzyme. The two preparations have similar specific activities with poly(C) as template and similar initiating capacities with both poly(C) and Qβ RNA as template. However, the control enzyme has 12 times as much EF-Tu·GDP binding activity and 19 times as much EF-Ts exchange activity as the "crosslinked" enzyme. The possibility that the activity seen with the "crosslinked" enzyme is due to the presence of the small amount of contaminating uncrosslinked EF-Tu and EF-Ts is made unlikely by the fact that the "crosslinked" enzyme has only 0.09 molar equivalent of EF-Tu·GDP binding activity and 0.04 equivalent of EF-Ts activity for every equivalent of initiating capacity with poly(C) under conditions in which reinitiation is prevented (Table 1). The kinetics of Qβ RNA replication *in vitro* by "crosslinked" (○) and control (●) replicase are shown in Fig. 3. The incorporation due to the first round of synthesis is given by the dashed curves, which represent experiments in which aurintricarboxylic acid was added after initiation in order to prevent reinitiation (9). The two enzyme preparations are indistinguishable by these criteria: both are capable of approximately linear incorporation for at least 20 min and both are clearly capable of initiation, elongation, and termination of synthesis.

On the other hand, the enzyme formed from crosslinked

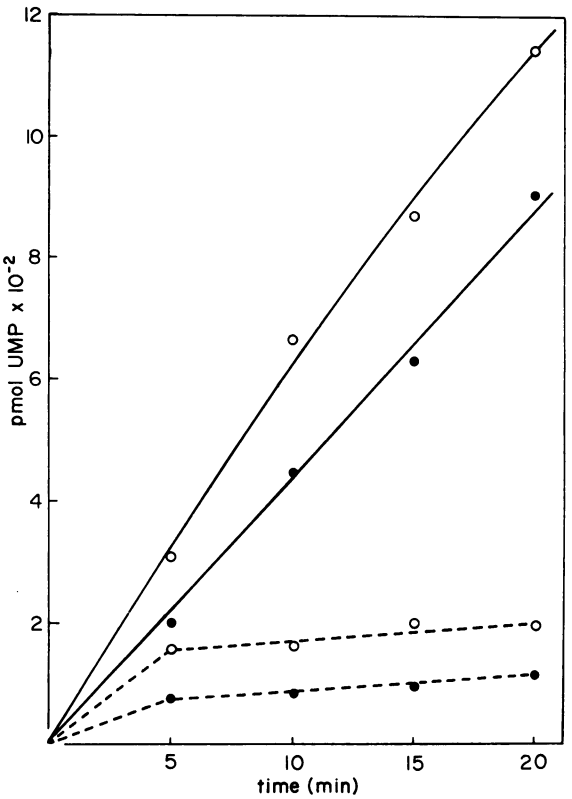

FIG. 3. Qβ RNA-dependent synthesis by "crosslinked" Qβ replicase and control replicase in the presence and absence of aurintricarboxylic acid. Solutions (0.32 ml) containing 80 mM Tris·HCl pH 7.5, 100 mM NaCl, 10 mM $Mg(OAc)_2$, 1 mM EDTA, 1 mM GTP, 1 mM ATP, 0.1 mM dithioerythritol, 1.4 μg of host factor, 42 μg of Qβ RNA, and either 4.0 μg of native replicase (●) or 3.5 μg of "crosslinked" replicase (○) were incubated for 5 min at 36°. They were then chilled to 2° and either aurintricarboxylic acid to 10 μM (dashed lines) or H_2O (solid lines) was added followed by ATP and GTP to a final concentration of 1.8 mM, CTP to 0.8 mM, and [^{3}H]UTP (34 cpm/pmol) to 0.2 mM. The reaction mixtures were incubated at 36°. Aliquots (50 μl) were removed at indicated times to 50 μl of cold 40 mM Tris·HCl pH 7.4, 120 mM NaCl, 20 mM EDTA and precipitated with trichloroacetic acid and filtered as described by Kamen (11). Backgrounds of 7 pmol of UMP were subtracted.

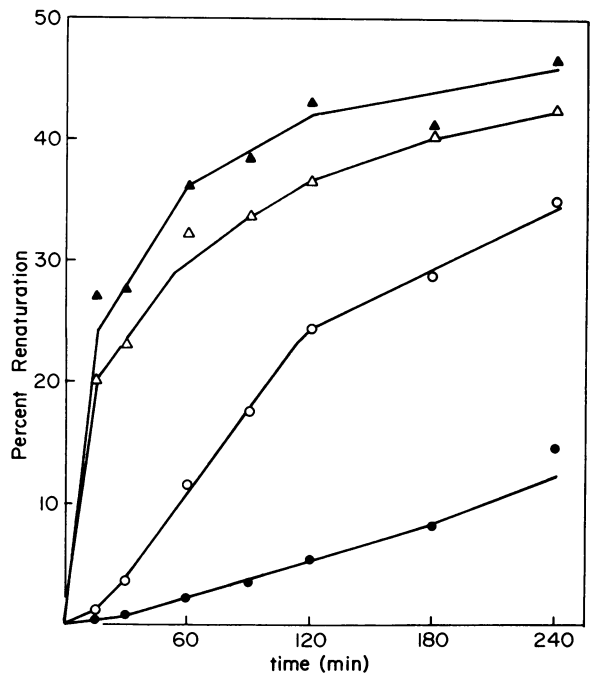

FIG. 4. Renaturation of denatured "crosslinked" Qβ replicase and control Qβ replicase in the presence and absence of GDP. Samples of "crosslinked" replicase (0.23 mg/ml) (△,▲) and control replicase (0.22 mg/ml) (○,●) were denatured in 8 M urea and renatured by dilution into high-salt buffer at 21° as described previously (8) in the presence (filled symbols) and absence (open symbols) of 0.5 mM GDP. Samples were removed and assayed for poly(G) polymerase activity at the times shown. Percent renaturation was determined by diluting nondenatured enzymes to the same concentrations in the same buffer with and without GDP and assaying at each of the times shown.

EF-Tu·Ts was found to be quite different from control replicase with respect to the kinetics of renaturation and the sensitivity of the renaturation to GDP (Fig. 4). The "crosslinked" replicase (△) renatures much faster than control enzyme (○), which is consistent with the stimulation in rate of renaturation provided by crosslinked EF-Tu·Ts (Fig. 1). Furthermore, while untreated Qβ replicase renatures very slowly in the presence of 0.5 mM GDP (●), the rate of recovery of the enzyme in which the GDP-binding site on EF-Tu has been inactivated by crosslinking to EF-Ts is not affected by the same concentration of GDP (▲). This observation provides further evidence that the crosslinked EF-Tu·Ts actually replaces EF-Tu and EF-Ts in this enzyme preparation.

DISCUSSION

The discovery that Qβ replicase contains protein synthesis elongation factors EF-Tu and EF-Ts (3) constituted the first case of polypeptides involved in one biosynthetic process being utilized in a different kind of biosynthetic process after phage infection. Our experiments have been designed to determine whether the functions performed by EF-Tu and EF-Ts in the RNA synthetic reaction are derived from those they perform in protein synthesis. EF-Tu can be assayed for catalysis of three reactions related to its function in the binding of aminoacyl-tRNA to ribosomes: binding of GTP or GDP, binding of aminoacyl-tRNA, and protein synthesis *in vitro*. In addition, EF-Ts can be assayed for catalysis of exchange of radioactively labeled GDP with EF-Tu·GDP at 0° (4). The possibility of the involvement of the aminoacyl-tRNA binding site in the RNA synthetic reaction has previously been eliminated (5, †). In this paper we demonstrate that the EF-Tu and EF-Ts can be inactivated for their nucleotide binding and exchange activities but can still play their normal role in transciption of both synthetic templates and Qβ RNA by Qβ replicase.

Covalent crosslinking of the EF-Tu to the EF-Ts results in the loss of the former's ability to bind guanine nucleotides and the latter's ability to catalyze GDP exchange with EF-Tu·GDP. The loss of activities is unlikely to be due simply to the treatment with dimethyl suberimidate, since treatment of the individual elongation factors does not result in loss of activity. EF-Tu·Ts crosslinked together stimulates the renaturation of denatured Qβ replicase more effectively than untreated EF-Tu·Ts or elongation factors treated separately with crosslinker. This provides strong support for our earlier suggestion that it is the EF-Tu·Ts complex that is active in the renaturation of the enzyme†. The stimulation of renaturation by the crosslinked product is insensitive to inhibition by GDP, a nucleotide which separates EF-Tu·Ts into EF-Tu·GDP and EF-Ts (4), further supporting the conclusion

that the crosslinked EF-Tu·Ts is the form that is active in the renaturation of the enzyme.

The observation that crosslinked EF-Tu·Ts stimulated renaturation of Qβ replicase under conditions in which the individual elongation factors did not (in the presence of GDP and kirromycin), provided us with an assay for crosslinked EF-Tu·Ts. We used this assay to partially purify the crosslinked product from a crude crosslinked postribosomal supernatant. The activity was found to elute from Sephadex G-100 ahead of GDP binding activity (EF-Tu, molecular weight = 45,000). The major protein found in the stimulatory fraction migrated on sodium dodecyl sulfate polyacrylamide gels with a molecular weight of about 80,000 (EF-Tu·Ts, molecular weight = 80,000). It crossreacted with anti-EF-Tu and anti-EF-Ts, indicating that crosslinked EF-Tu·Ts was in fact the activity that was purified. The fact that crosslinked EF-Tu·Ts can be isolated from a crude preparation of crosslinked proteins by virtue of its ability to replace EF-Tu and EF-Ts during renaturation of denatured Qβ replicase provides strong support for the contention that the crosslinked EF-Tu·Ts is active in Qβ replicase.

After a denatured sample of Qβ replicase was renatured in the presence of this crosslinked EF-Tu·Ts and then purified, the modified replicase was studied. The enzyme in which the covalently linked EF-Tu·Ts complex replaces the individual elongation factors has approximately the same specific activity, initiation capacity with poly(C) and Qβ RNA, and antigenicity with anti-EF-Tu and anti-EF-Ts as control enzyme. However, it is severely deficient in the activities catalyzed by the individual elongation factors (Table 1). The data show that a molar excess of phage-induced activities is present over known host-specified activities. It has already been established that although there is subunit exchange during the purification of Qβ replicase (1, 2), in the *in vitro* RNA synthetic mixture, subunit exchange is very slow (10). Therefore, a molar quantitation of the number of RNA chains initiated by the replicase may be compared with the number of moles of [^{3}H]GDP bound by the same preparation to nitrocellulose filters, since Qβ replicase and EF-Tu each bind 1 mole of [^{3}]HGDP per mole of protein (Table 1 and ref. 4). After reconstitution of replicase with crosslinked EF-Tu·Ts, the RNA initiating capacity is in manyfold molar excess over GDP binding, while native replicase binds greater than 1 mole of GDP per mole of RNA initiating capacity. Thus, the possibility that the GDP/GTP binding site of EF-Tu is involved in initiation of synthesis by Qβ replicase is made extremely unlikely. Since binding of GTP to EF-Tu is a prerequisite for all known EF-Tu activities (4), presumably the remaining protein synthetic functions cannot be performed by the crosslinked complex.

The data presented here demonstrate that the known protein synthesis-related activities of the individual elongation factors are not required for Qβ RNA replication. Two possible roles of the EF-Tu·Ts in Qβ replicase remain: first, the EF-Tu·Ts complex might have an activity in the uninfected host which is yet to be identified and is being exploited by the phage. Alternatively, EF-Tu·Ts, once it becomes part of Qβ replicase might acquire an activity which is not used by the host. The observation that EF-Tu·Ts performs its function normally in replicase even after treatment with a bifunctional crosslinking reagent, suggests the possibility that it plays a purely structural role, perhaps involving constraint of the phage-coded subunit in an active conformation.

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