Function and Structure in Phage Qβ RNA Replicase

ASSOCIATION OF EF-Tu·Ts WITH THE OTHER ENZYME SUBUNITS*

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Qβ replicase is a complex of four nonidentical subunits readily dissociable into two subcomplexes: 30 S ribosomal protein S1 and the phage-coded polypeptide (Subunits I + II) and protein synthesis elongation factors EF-Tu and EF-Ts (Subunits III + IV). The affinity of the two subcomplexes for one another increases with increasing ionic strength. The enzyme is capable of initiation of RNA synthesis with synthetic templates only when in the low ionic strength conformation. Elongation of initiated polynucleotide chains is not affected by ionic strength.

Addition of Qβ RNA to the enzyme also alters its quaternary structure: the EF-Tu·Ts cannot be covalently attached to the other enzyme subunits with bifunctional cross-linking reagents in the presence of RNA. This conformational change is not influenced by ionic strength. The addition of Qβ RNA to the enzyme, does not result in the release of EF-Tu·Ts from the other enzyme subunits: whereas free EF-Tu·Ts binds GDP independently of salt concentration, this binding by Qβ replicase is sensitive to high ionic strength and remains so in the presence of Qβ RNA. Furthermore, RNA does not allow the release of EF-Ts from EF-Tu by GTP as measured by sensitivity of EF-Ts activity to N-ethylmaleimide.

Qβ phage RNA replicase (nucleoside triphosphate:RNA nucleotidyltransferase (RNA dependent)) is composed of four nonidentical subunits, three of which are coded for by the host, Escherichia coli (1, 2). The host polypeptides are part of the protein synthetic machinery in uninfected cells: the largest (Mr = 70,000) has recently been found to be 30 S ribosomal protein S1 (3, 4). The smaller two are protein synthesis elongation factors EF-Tu and EF-Ts (Mr = 45,000 and 35,000, respectively) (5). The phage-coded subunit (Mr = 65,000) must be responsible for the high specificity of the enzyme for its natural template (6), since the other subunits are host-coded and are found in RNA bacteriophage RNA replicases with different template specificities (7).‡ The phage-coded subunit is capable of performing the elongation reaction on synthetic templates in the absence of the host-coded subunits (8). Subunit I is required only for initiation of transcription of Qβ RNA (9), while EF-Tu and EF-Ts are necessary for initiation of transcription of synthetic templates (8) as well as Qβ RNA (10).

The enzyme is composed of one each of the four subunits (11). It can be further subdivided into two smaller complexes composed of the two larger subunits and EF-Tu·Ts (1, 2, 11). Using the technique of intramolecular protein cross-linking we have recently found that the two smaller complexes are associated in a different, “tighter,” complex at high ionic strength, than at low ionic strength (11). In addition, we have shown (8) that both GDP binding by EF-Tu and [3H]GDP exchange catalyzed by EF-Ts are inhibited by increasing ionic strength when these polypeptides form part of the replicase enzyme, although with the individual factors these activities are not influenced by salt concentration (8). We report here that increasing ionic strength results in a concomitant decrease in the ability of the enzyme to initiate transcription of synthetic templates, but not elongation of initiated RNA molecules.

There is considerable evidence that addition of RNA to Qβ replicase alters the quaternary structure of the enzyme: when either synthetic templates (1, 8) or Qβ RNA (2) are mixed with the enzyme (with or without GTP) and the mixture is subjected to zone sedimentation on sucrose gradients, the EF-Tu and EF-Ts are separated from the other enzyme subunits which remain bound to the RNA. Furthermore we have shown (11) that when Qβ RNA is added to the enzyme the EF-Tu·Ts can no longer be cross-linked to the other enzyme subunits by dimethyl suberimidate. These results suggest the possibility that the presence of RNA results in the release of EF-Tu and EF-Ts from the enzyme. In this paper we present evidence that the conformational change induced by Qβ RNA...
**RESULTS**

Inhibition of Qβ Replicase Activity by Salt—In order to determine the effect of ionic strength on the rate of initiation of Qβ replicase on synthetic templates, the enzyme was incubated at 25° with poly(A,C) and GTP (the initiating nucleoside triphosphate (18)) at a variety of salt concentrations. Samples were periodically removed to tubes containing aurintricarboxylic acid, a reagent that prevents Qβ replicase initiation but not elongation (19). [3H]GDP was then added and the reaction was incubated for 5 min at 25°. (This is approximately 3 times as long as required to complete the chains.) Fig. 1 demonstrates that the greatest initial rate of initiation was found at the lowest ionic strength tested (no added NaCl). There is an inverse relationship between ionic strength and rate of initiation of Qβ replicase on this template.

The effect of ionic strength on the rate of elongation was also measured: ample time (5 min at 25°) for complete initiation on poly(A,C) was allowed at low ionic strength before the addition of NaCl. (Formation of aurintricarboxylic acid-resistant initiation complexes is complete within 1 min at 25°.) The [3H]GTP was then added to permit elongation and sequential samples were removed and precipitated. Again reinitiation was prevented by the addition of aurintricarboxylic acid with the UTP. The results (Fig. 2) show that there was little, if any, effect of ionic strength on the elongation rate.

Correlation of Initiation Inhibition with Alteration of Enzyme Quaternary Structure—Since several recent lines of evidence indicate that the replicase subunits associate differently with varying ionic strength (1, 8, 11), it is important to determine whether the change in initiation activity correlates with the change in enzyme quaternary structure. A convenient assay for this alteration in subunit association is the binding of [3H]GTP to EF-Tu. Although this binding is not normally affected by ionic strength, the binding of [3H]GTP to EF-Tu when it is a subunit of Qβ replicase is strongly inhibited by high ionic strength (8). Clearly this inhibition must result from interaction with the other replicase subunits and can be

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1. T. Blumenthal, unpublished observations.
assumed to reflect the nature of the association between the polypeptides. Table I shows that, although the binding of \(^{3}H\)GTP by EF-Tu-Ts is not affected by ionic strength, the binding of \(^{3}H\)GTP by Q\(\beta\) replicase and the initiation on poly(A,C) by the enzyme are lost in parallel. Thus, the alteration in enzyme quaternary structure resulting in loss of GTP-binding activity correlates with the loss of RNA synthesis activity with increasing ionic strength.

**Lack of Effect of Ionic Strength on Dissociation of Q\(\beta\) Replicase by Q\(\beta\) RNA**—The amount of 215,000 molecular weight complex formed by cross-linking the four Q\(\beta\) replicase subunits together with dimethyl suberimidate in the presence or absence of RNA was measured at varying ionic strengths. It was found (Table II) that, although increased amounts of this complex were formed at higher ionic strengths, the same percentage of the complex was lost in each case when an equimolar quantity of Q\(\beta\) RNA was added. This suggests that the dissociating effect of RNA is not related to the dissociating effect of low ionic strength.

**Protein Synthesis Elongation Factors are Not Released from Q\(\beta\) Replicase in the Presence of Q\(\beta\) RNA**—Because binding of GTP or GDP to EF-Tu-Ts is not sensitive to ionic strength changes while binding of these nucleotides to EF-Tu when it is a part of Q\(\beta\) replicase is (8), this assay can be used to determine whether the EF-Tu is enzyme-associated. When equimolar Q\(\beta\) RNA is added to the enzyme it is found that the binding of \(^{3}H\)GDP is still inhibited by increased salt concentration (Fig 3). Thus, under these conditions it must remain associated with the rest of the enzyme. We do not have an explanation for the (reproducible) observation that GDP binding is less sensitive to salt concentration than is GTP binding (compare Table I and Fig. 3).

The question of whether the EF-Tu-Ts is released by interaction of the enzyme and RNA can also be answered by determining if the EF-Ts can be separated from the EF-Tu by GTP, since free EF-Tu-Ts is dissociable by GTP, while EF-T\(\alpha\)-Ts in Q\(\beta\) replicase is not (8). EF-Ts contains an essential sulfhydryl group which is protected from inactivation by N-ethylmaleimide in the EF-Tu-Ts complex (20). Addition of GDP to EF-Tu (along with Q\(\beta\) RNA and host factor, an additional host-coded protein required for in vitro Q\(\beta\) RNA replication) to EF-Tu-Ts dissociates the complex and renders the EF-Ts sensitive to N-ethylmaleimide (Table III). However, when the same components are added to Q\(\beta\) replicase, the EF-Ts activity is not inhibited by N-ethylmaleimide (Table III), indicating that addition of RNA and host factor does not result in release of EF-Tu-Ts. We cannot explain the small but reproducible stimulation of the EF-Ts activity by N-ethylmaleimide.

**DISCUSSION**

The association of Q\(\beta\) replicase subunits has been shown to be sensitive to ionic strength: EF-Tu-Ts separates from the complex of Subunits I + II during glycerol gradient centrifugation in low ionic strength buffer (1); significant amounts of elongation factors bound to the other replicase subunits after treatment with chemical cross-linkers are found only when treated at high ionic strengths (11). Moreover, the poly(C)-dependent poly(G) polymerase isolated from phage \(\Phi\)-infected *Escherichia coli* has been shown to be inhibited by increasing salt concentration (21). We demonstrate here that high ionic strength also alters the functioning of Q\(\beta\) replicase. Initiation of transcription of synthetic templates is inhibited by salt, while elongation of already initiated polynucleotide chains is not. A consideration then, is whether this inability to initiate is related to the type of association of EF-Tu and EF-Ts with the two larger subunits. If it is, we would expect to find the two to be correlated as ionic strength is changed. A convenient assay for subunit association is the binding of GTP to EF-Tu, since this binding is only sensitive to ionic strength when the EF-Tu is a subunit of Q\(\beta\) replicase (8). The binding is lost in parallel with the loss of initiation activity. We suggest that this correlation of the two activities is a reflection of their common dependence on the tightness of binding of the protein synthesis elongation factors to the other enzyme subunits. These results

![Table I](https://www.jbc.org/content/250/5/2742)

**TABLE I**

| Ionic strength | GTP Binding | Initiation activity |
|---------------|-------------|---------------------|
|               | EF-Tu-Ts    | Q\(\beta\) replicase |
| 0.075         | 100         | 100                 |
| 0.125         | 101         | 64                  |
| 0.175         | 100         | 39                  |
| 0.250         | 95          | 18                  |

![Table II](https://www.jbc.org/content/250/5/2742)

**Table II**

**Inhibition of Q\(\beta\) replicase complex formation by Q\(\beta\) RNA at varying ionic strengths**

Q\(\beta\) replicase (2.3 \(\mu\)M) was cross-linked in the presence and absence of an equimolar concentration of Q\(\beta\) RNA with dimethyl suberimidate as described previously (11). The cross-linked complexes were separated by electrophoresis on 5% polyacrylamide-SDS gels and the amount of 215,000 molecular weight complex formed was estimated from gel scans as described (11).

| Ionic strength | Amount 215,000 molecular weight complex formed |
|---------------|---------------------------------|
| 0.1           | 41                              |
| 0.4           | 114                             |
| 1.0           | 148                             |

![Table III](https://www.jbc.org/content/250/5/2742)

**Table III**

**Inhibition of EF-Ts activity by N-Ethylmaleimide**

EF-Tu-Ts (5 x 10^{-7} M) and Q\(\beta\) replicase (3 x 10^{-4} M) were treated with N-ethylmaleimide (or an equivalent volume of ethanol) in the presence of equimolar Q\(\beta\) RNA and host factor with and without 20 \(\mu\)M GTP as described under "Experimental Procedure." After quenching the reactions with dithioerythritol, the preparations were assayed for EF-Ts activity. In this assay the EF-Tu alone bound 192 cpm.

| Additions          | Cpm bound (% of control) |
|-------------------|--------------------------|
| EF-Tu-Ts           |                          |
| Q\(\beta\) replicase|                          |
| Ethanol           | 2491                     |
| N-Ethylmaleimide  | 2209 (91%)               |
| GTP + N-ethylmaleimide | 756 (31%)           |
EF-Tu-Ts in Qβ Replicase

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