EFFECT OF NH_4^+ AND K^+ ON THE ACTIVITY OF THE RIBOSOMAL SUBUNITS IN THE EF-G- AND EF-T-DEPENDENT GTP HYDROLYSIS

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Summary: E. coli 50S ribosomal subunits show in the absence of 30S subunits and at low NH_4^+ or K^+ high turnover activity in EF-G-dependent GTP hydrolysis which is inhibited by increasing concentrations of monovalent cations. At 80 mM NH_4^+ or K^+ this activity is already 70-80% inhibited. This effect is reversed by 30S which are stimulatory with an optimum at about 80 mM for NH_4^+ and 20-40 mM for K^+. At low NH_4^+ or K^+ (<5 mM) stimulation by 30S of maximal 50S activity depends on the [EF-G]/[50S]. Unlike EF-G, EF-T does not show any Phe-tRNA-dependent GTPase activity with 50S alone even at low concentrations of NH_4^+ or K^+.

Since the localization of the ribosomal center for EF-G-dependent GTP hydrolysis on the 50S subunit (1,2) the role of the two subunits in the expression of turnover activity of EF-G-dependent GTPase has presented still unsolved aspects. Results have been published which indicated that the 50S subunit alone was able to support a considerable portion of the turnover activity of the EF-G-dependent GTPase reaction obtained in the presence of 30S (3,4). Other experiments indicated that association of the ribosomal subunits was the basic requirement for GTPase activity of EF-G (5-7), as already observed by Conway and Lipmann (8). Under optimal conditions the small GTPase activity measured with 50S alone depended almost entirely on contaminating 30S subunits. In the presence of methanol, however, 50S alone supported an EF-G GTPase activity far beyond that which could be attributed to 30S contamination (6). These differences have induced us to extend these studies by investigating several additional parameters which could regulate turnover activity of ribosomal EF-G GTPase. We now show in this communication that the EF-G GTPase reaction displays high activity

with the 50S subunit alone at low NH_4^+ or K^+ and that these cations inhibit the 50S-dependent activity at concentrations optimal for protein biosynthesis in vitro.

MATERIALS AND METHODS

Pure EF-G, EF-T (EF-Tu + EF-Ts) and ribosomes from E. coli B $T2^r$ or A19 were prepared essentially as described (9). Ribosomal subunits were isolated by sucrose density gradient centrifugation at 0.5 mM $MgCl_2$ using a Spinco 15 Ti zonal rotor. The 30S were 98% pure and the 50S 96-97%, as measured by sedimentation in analytical sucrose density gradients. Poly(U)-dependent Phe-tRNA binding and poly(U)-dependent polyphenylalanine synthesis indicated that the 50S were contaminated with 4-6% 30S active in these reactions. Our results were, however, not corrected for this contamination because the heterogeneity in the different functions of the 30S does not allow an accurate estimate (10,11). One A_{260} unit was taken to represent 25 pmoles 70S, 39 pmoles 50S or 67 pmoles 30S particles (12,13). γ-[^{32}P]GTP was prepared by the method of Glynn and Chappell (14,15) and purified by elution from DEAE-cellulose in bicarbonate form with a gradient of triethylammonium bicarbonate buffer (16). Protein determinations were done according to Lowry et al. (17) using crystalline bovine serum albumin as the standard. $tRNA^{Phe}$ was obtained 40% pure from commercial preparations of tRNA (Schwarz) (18). EF-G and EF-T ribosome-dependent GTPases were assayed as reported using 14 mM Mg^{2+} throughout this work (19). Monovalent cations present in the ribosome solutions were not removed prior to the assays to avoid possible destabilization phenomena. Therefore, the lowest concentration of K^+ or NH_4^+ tested was 2 mM. To overcome effects due to functional heterogeneity and to obtain optimal activity with the resulting 70S ribosome, 30S subunits were present in the assays in the excess needed for maximal stimulation of the 50S subunits (6,20). This was approximately 7-fold for EF-G and 2-fold for EF-T.

For the details of the single experiments see legends.

RESULTS

Effect of NH_4^+ and K^+ on the EF-G GTPase activity of the ribosomal subunits

Fig. 1 shows that EF-G exhibits a significant GTPase activity with the 50S alone in the absence of added monovalent cations and at 14 mM Mg^{2+}. Increasing amounts of NH_4^+ or K^+ caused progressive inhibition of the reaction. In the presence of saturating concentrations of 30S subunits a different effect of the same cations was observed. In fact, the inhibition by NH_4^+ and K^+ was reversed and these cations, particularly NH_4^+, became

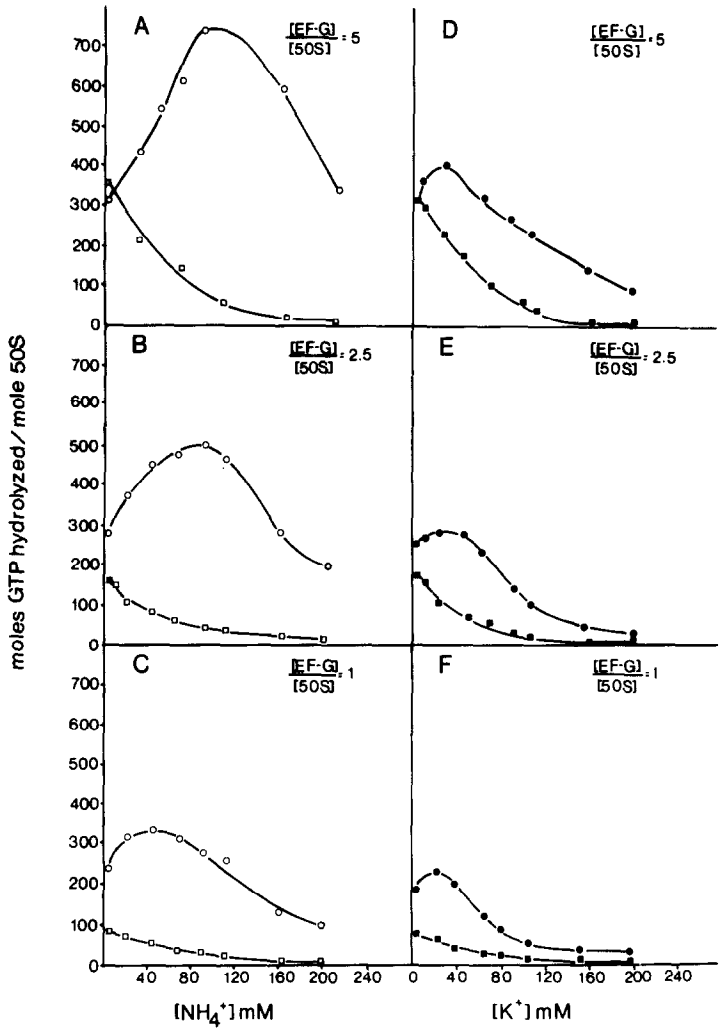

Fig. 1. Effect of NH_4^+ and K^+ on the EF-G-dependent GTPase activity of 50S or 50S plus 30S. The 75 µl reaction mixture contained: 20 mM Tris-HCl, pH 7.8 - 14 mM $MgCl_2$ - 14 mM 2-mercaptoethanol - 2% glycerol (from ribosomes) - 25 nmoles γ-[^{32}P]GTP (2-3 Ci/mole) - 10 pmoles 50S ribosomal subunits - 70 pmoles 30S subunits when present, NH_4Cl or KCl as indicated. 50 pmoles of EF-G were added in A and D, 25 pmoles in B and E and 10 pmoles in C and F. After incubation for 10 min at 30°C, the GTPase activity was measured as the amount of $^{32}P_i$ liberated (27). Results in all figures were not corrected for contamination of the 50S subunits by 30S (see Methods). The 30S blank was between 2.5 and 15 pmoles GTP hydrolyzed/mole 30S depending on $[NH_4^+]$ or $[K^+]$ and [EF-G]/[50S]. These values could be entirely attributed to the contaminating 50S (see Methods) and were subtracted.

□——□ 50S with NH_4^+
■——■ 50S with K^+
○——○ 50S plus 30S with NH_4^+
●——● 50S plus 30S with K^+

stimulatory at low concentrations. Optimal activity was reached with NH_4^+ at ~80 mM and was approx. twice as high as with K^+ which maximally stimulated around 30 mM. Both the degree of stimulation by 30S at $[NH_4^+]$ or $[K^+] < 5$ mM and maximal activity of EF-G GTPase with 50S alone depended on the EF-G to 50S molar ratio. With a ratio of 5 the 30S had essentially no stimulatory activity without monovalent cations, while by decreasing the molar ratio stimulation by the 30S became evident. At the ratio of 1, which is approximately that found in E. coli cell extracts (21), stimulation by 30S was about threefold. Maximal 50S activity compared with maximal 70S activity was reduced from 50 to 25% with NH_4^+ and from 80 to 35% for K^+ by decreasing the [EF-G]/[50S] from 5 to 1. As already reported in previous work performed at 80 mM NH_4^+ (6), EF-G-50S GTPase activity was practically negligible at high concentrations of NH_4^+ or K^+. All of the little EF-G GTP hydrolysis observed in the presence of the 30S alone could be explained by the 50S contaminating the 30S preparations (see Methods). In the absence of EF-G, we were not able to detect any GTPase activity of the ribosomal subunits.

As shown in Fig. 2, with 20% (v/v) methanol and both ribosomal subunits, optimal activity was obtained around 50 mM NH_4^+ or K^+. Only a slight decrease of activity was observed above the optimum. The 50S system now needed 30 mM NH_4^+ or around 50 mM K^+ for optimal activity. GTPase could be stimulated by 30S subunits over the whole range of NH_4^+ concentrations. In the case of K^+, however, the 50S system was stimulated by 30S only at salt concentrations where the 50S-EF-G GTPase activity was suboptimal.

Effect of NH_4^+ and K^+ on the ribosomal GTPase activity of EF-T

The EF-T-dependent ribosomal GTPase reaction has previously been shown to require both ribosomal subunits in work performed at 50 mM (19) or 80 mM NH_4^+ (22). Fig. 3 shows that, unlike EF-G, this requirement held true

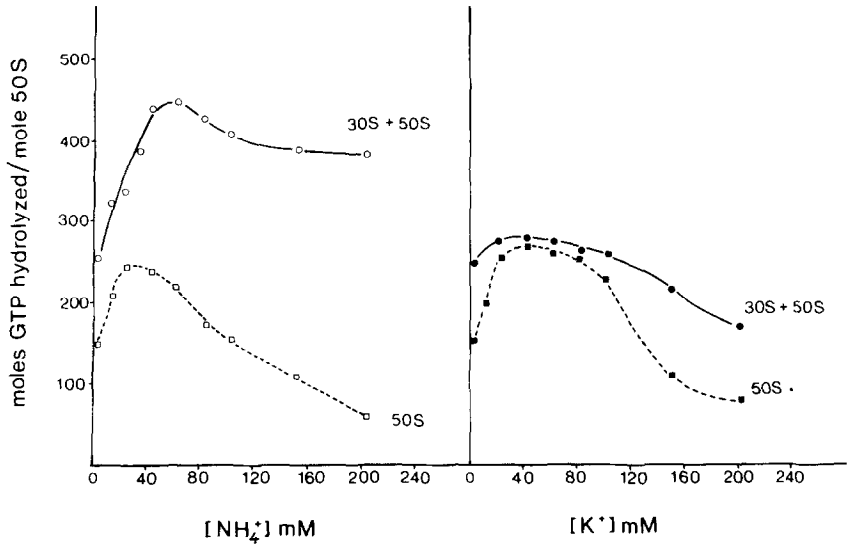

Fig. 2. Effect of NH_4^+ and K^+ on the EF-G-dependent GTPase of ribosomal subunits in the presence of methanol. Conditions as described in Fig. 1 except for the presence of 20% (v/v) methanol. In these experiments an [EF-G]/[50S] of 5 was used.

□------□ 50S with NH_4^+ ○——○ 50S plus 30S with NH_4^+
■- - - -■ 50S with K^+ ●——● 50S plus 30S with K^+

at all NH_4^+ or K^+ concentrations tested. With both subunits increasing [NH_4^+] induced an inhibition with apparent sigmoidal shape. K^+ showed a somewhat sharper decrease in the first part of the curve. If 70S ribosomes instead of individual subunits were added to the assay system, an NH_4^+ optimum between 40 and 50 mM was observed (not shown). In the presence of 20% (v/v) methanol (Fig. 3B), NH_4^+ or K^+ stimulated at low concentrations and were required for maximal activity with an optimum around 50 mM for NH_4^+ and 20 mM for K^+. 50S alone again did not show any activity.

No Phe-tRNA- or ribosome-independent GTPase activity was observed with our EF-T preparations in all conditions tested in this work.

DISCUSSION

Our results show that 50S subunits alone are capable to support EF-G GTPase activity at concentrations of monovalent cations far below the

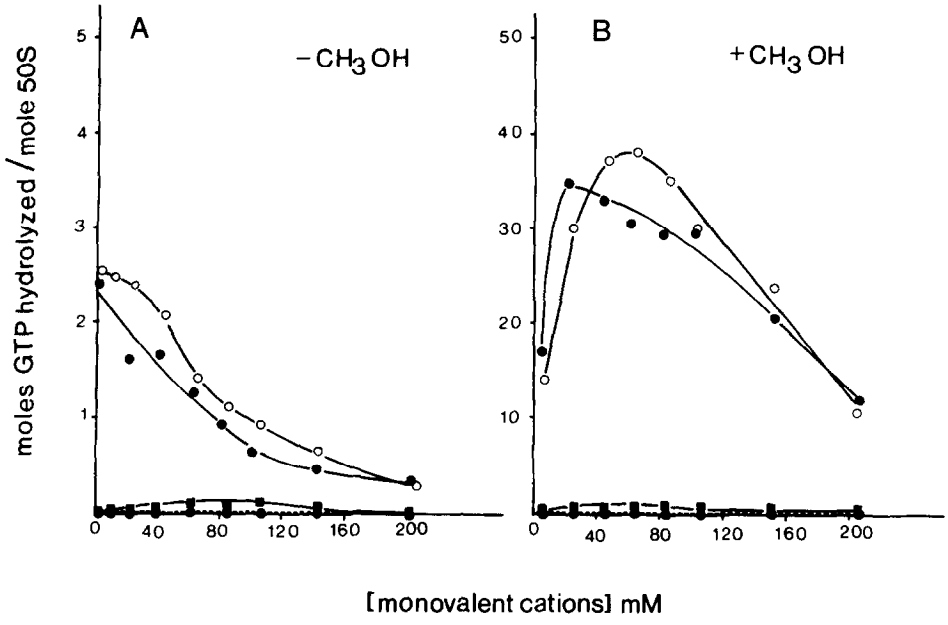

Fig. 3. Effect of NH_4^+ and K^+ on the EF-T-GTPase in the absence (A) and presence (B) of 20% (v/v) methanol. The assay system contained in 75 µl: 20 mM Tris-HCl, pH 7.8 - 14 mM $MgCl_2$, about 0.5% glycerol (from ribosomes), 5 pmoles of 50S subunits when present, 10 pmoles of 30S where indicated, 60 pmoles of EF-T, 80 pmoles of $[^{14}C]$Phe-tRNA^{Phe} when present, 3 µg of poly(U) and 100-120 pmoles of γ-$[^{32}P]$GTP (~1000 Ci/mole) in the absence of methanol or 1000-1200 pmoles of γ-$[^{32}P]$GTP (~100 Ci/mole) in its presence. The reaction mixture was incubated for 2 min at 30°C and the GTPase activity measured as the amount of $[^{32}P_i]$ liberated (27).

O——O NH_4Cl with 50S plus 30S
●——● KCl with 50S plus 30S
■——■ NH_4Cl or KCl with 50S alone
●- - -● Controls without Phe-tRNA^{Phe} in the presence or absence of 50S and 30S

optimum for *in vitro* protein synthesis. In fact, at the Mg^{2+} concentration used in these experiments (14 mM) optimal activity in poly(U)-directed polyphenylalanine synthesis is obtained with $[NH_4^+]$ or $[K^+]$ above 100 mM (23,24) which in our system strongly inhibit EF-G activity with 50S alone. It is interesting that optimum growth conditions for *E. coli* are observed with an intracellular total K^+ content of 200-300 mM (25,26). With NH_4^+, unlike other reports (3,4), activity of 50S alone was very low already at 80 mM if corrected for contaminating 30S and negligible at con-

centrations above 150 mM when compared with the activity obtained by addition of 30S subunits.

The molar concentration of EF-G relative to that of the 50S ribosome represents a critical parameter in this reaction. In fact, strong stimulation by 30S subunits with low $[NH_4^+]$ or $[K^+]$ is already caused by a change of [EF-G]/[50S] from 5 to 2.5 (Fig.1). The observation that decreasing the EF-G to 50S molar ratio induces a much stronger dependence on the 30S subunit of the EF-G GTPase activity even at low concentrations of monovalent cations indicates a greater affinity of EF-G for the 70S ribosome. A detailed study of the kinetic properties of the EF-G-dependent GTPase with the 50S alone and with the 50S plus 30S will perhaps answer the many open questions suggested by this work.

In contrast to the results obtained with EF-G, EF-T GTPase needs both ribosomal subunits to express its activity even at concentrations of monovalent cations at which 50S-dependent GTPase of EF-G is particularly active.

The results of this communication point out the great importance of the ionic conditions for the expression of the turnover activity of the EF-G-dependent GTPase with the 50S subunit alone and also the central role of the 30S subunit in the regulation of this reaction.

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