a specific channel, within a machinery that transduces metabolic energy into unidirectional transfer of the growing chain.

References

Adelman, M. R., Sabatini, D. D., & Blobel, G. (1973) *J. Cell Biol. 56*, 206–229.
Aronson, A. (1966) *J. Mol. Biol. 15*, 505–514.
Blobel, G., & Sabatini, D. D. (1971) in *Biomembranes* (Manson, L. A., Ed.) Vol. 2, pp 193–196, Plenum Press, New York.
Blobel, G., & Dobberstein, B. (1975) *J. Cell Biol. 67*, 835–851.
Carraway, K. L. (1975) *Biochim. Biophys. Acta 415*, 319–410.
Chua, N.-H., Blobel, G., Siekevitz, P., & Palade, G. E. (1976) *J. Cell Biol. 71*, 497–514.
Davis, B. D., & Mingioli, E. S. (1950) *J. Bacteriol. 60*, 17–28.
Devillers-Thiery, A., Kindt, T., Scheele, G., & Blobel, G. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 5016–5020.
Furano, A. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4780–4784.
Hirashima, A., & Kaji, A. (1972) *Biochemistry 11*, 4037–4044.
Inouye, H., & Beckwith, J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1440–1444.
Inouye, S., Wang, S., Sekizawa, J., Halegoua, S., & Inouye, M. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1004–1008.
Katz, F. N., Rothman, J. E., Lingappa, V. R., Blobel, G., & Lodish, H. F. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3278–3282.
Kung, H. F., Treadwell, B., Spears, C., Tai, P.-C., & Weissbach, H. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3217–3221.
Milstein, C., Brownlee, G., Harrison, T., & Mathews, M. (1972) *Nature (London), New Biol. 239*, 117–120.
Palade, G. E. (1975) *Science 189*, 347–358.
Randall, L. L., & Hardy, S. J. S. (1977) *Eur. J. Biochem. 75*, 43–53.
Rothman, J. E., & Lodish, H. F. (1977) *Nature (London) 269*, 775–780.
Sabatini, D. D., & Kreibich, G. (1976) in *The Enzymes of Biological Membranes* (Martonosi, A., Ed.) Vol. 2, pp 531–579, Plenum Press, New York.
Schechter, I., McKean, D. J., Guyer, R., & Terry, W. (1975) *Science 188*, 160–162.
Smith, W. P., Tai, P.-C., Thompson, R. C., & Davis, B. D. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 2830–2834.
Smith, W. P., Tai, P.-C., & Davis, B. D. (1978a) *Proc. Natl. Acad. Sci. U.S.A. 75*, 814–817.
Smith, W. P., Tai, P.-C., & Davis, B. D. (1978b) Abstracts, Annual Meeting of the American Society of Microbiology, Las Vegas, Nev., May 17, 1978, p 144.
Smith, W. P., Tai, P.-C., & Davis, B. D. (1978c) *Proc. Natl. Acad. Sci. U.S.A. 75*(12), in press.
Toneguzzo, F., & Ghosh, H. P. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 715–719.

Cation-Induced Regulatory Mechanism of GTPase Activity Dependent on Polypeptide Initiation Factor 2†

Patrice Beaudry,‡ Gernot Sander,§ Marianne Grunberg-Manago,* and Pierre Douzou¶

ABSTRACT: Initiation factor IF-2 ribosome dependent GTP hydrolysis (uncoupled GTPase) presents a bell-shaped pH profile which is shifted by changes in ionic strength. At low ionic strength (I = 25 mM) the maximal hydrolytic activity occurs at pH 7.5; when the ionic strength is increased the pH optimum of the reaction is shifted toward more acidic values. Such behavior can be satisfactorily explained as the effect of an electrostatic potential developed by a neighboring polyanion, presumably RNA, on the catalytic site. The addition of fMet-tRNA^{fMet} or AcPhe-tRNA^{Phe} and messenger RNA (coupled GTPase) changes the ionic strength–pH characteristics of the reaction. Thus there is an effect, direct or indirect, of components located at the ribosomal P site. Investigation of the effect of neighboring polyanions on the catalytic activity of the factor-dependent ribosomal GTPases can be seen to provide information about their functional significance that is complementary to that gained from direct structural studies.

Ribosomes are essential elements of GTPase activities developed by initiation factor IF-2 as well as elongation factors EF-G and EF-Tu during *Escherichia coli* protein synthesis in vitro (Kolakofsky et al., 1969; Lelong et al., 1970; Dubnoff & Maitra, 1972; Stöffler & Wittmann, 1977; Grunberg-Manago et al., 1978).

GTP appears to interact directly with these factors, at least with the last two (Miller & Weissbach, 1977; Brot, 1977; Grunberg-Manago et al., 1978). However, at present, it is not clear whether the catalytic site for GTP hydrolysis is located on the ribosome or on the protein factor itself. For EF-Tu, it has been shown that the elongation factor is capable of developing GTP hydrolytic activity in the presence of the antibiotic kirromycin (Chinali et al., 1977). Isolated ribosomal protein L7/L12 also seems to be able to induce an elongation factor Tu dependent GTPase activity (Donner et al., 1978).

It has also been shown that EF-G and EF-Tu dependent GTPase activities were very sensitive to monovalent cation variations such as K^+ or NH_4^+ (Voigt et al., 1974; Arai &

† From the Institut de Biologie Physico-Chimique, 13 rue P. et M. Curie, 75005 Paris, and Laboratoire de Biochimie, Ecole Polytechnique, 91128 Palaiseau Cédex, France. *Received August 10, 1978.* This work was supported by the following grants: Centre National de la Recherche Scientifique [GR 18 and D.G.R.S.T. (79.7.1178 and 76.7.1186)], C.E.A., Ligue Nationale contre le Cancer (Comité de la Seine), and Fondation pour la Recherche Médicale Française. Dedicated to Edgar Lederer for his 70th anniversary.

* Address correspondence to the Institut de Biologie Physico-Chimique, Service de Biochimie.
‡ Present address: Institut de Biologie Physico-Chimique, Service de Biochimie.
§ Laboratoire de Biochimie, Ecole Polytechnique.
¶ Institut de Biologie Physico-Chimique, Service de Biospectroscopie.

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Kaziro, 1975). Many structural arguments indicate that the ribosomal sites for EF-G, EF-Tu, and IF-2 dependent GTP hydrolysis might be quite close to each other (Stöffler & Wittman, 1977; Grunberg-Manago et al., 1972).

In the present article we investigate the effect of pH and of monovalent cations (NH_4^+) on IF-2 dependent GTPase activity. The variations of enzymatic activity observed under these conditions can be interpreted by polyelectrolyte theory in its simplest form (Douzou & Maurel, 1977) and indicate that the catalytic site of the GTPase is dependent on a polyanionic microenvironment sensitive to pH changes and cation concentrations. This ribosome-dependent enzymatic reaction appears therefore to be controlled by a polyanionic microenvironment, possibly representing a regulatory mechanism. IF-2 dependent GTP hydrolysis is an essential step in the initiation of protein synthesis since it enables IF-2 to recycle (Grunberg-Manago et al., 1978). Thus, a regulatory step whereby cations modulate the IF-2 GTPase reaction is obviously important for the control of protein synthesis.

The positioning of ribosomal RNA with respect to the ribosomal site of the GTPases dependent on IF-2, EF-Tu, and EF-G factors, as well as the effect of components located at the P site, will also be discussed.

Materials and Methods

GTP was supplied by Miles Laboratories; (γ-^{32}P)GTP was from the Radiochemical Centre, Amersham (specific activity 17.6 Ci/mmol); magnesium acetate from Carlo Erba; ammonium chloride from Prolabo; and Tris-HCl from Sigma. Unpurified ^{3}H-fMet-tRNA (*E. coli*) was prepared as described previously (Lelong et al., 1970). Poly(A,U,G) was synthesized by polynucleotide phosphorylase as described by Godefroy-Colburn & Grunberg-Manago (1972). Poly(U) was obtained from Boehringer. Ac Phe-tRNA^{Phe} was prepared according to Haenni & Chapeville (1966).

Preparation of Ribosomes and Factors. Preparation of A-Type Ribosomes. E. coli extracts (strain MRE 600) were prepared by grinding with alumina; ribosomes were prepared by centrifugation of the extract at low and high speed. They were washed twice in high-salt (1.5 M NH_4^+) buffer according to the procedure of Dondon et al. (1974). The washed ribosomes were then purified on a 10–30% sucrose gradient by centrifugation for 17 h at 31 000 rpm in a Beckman T14 zonal rotor in the following buffer: 10 mM Tris-HCl (pH 7.5), 60 mM NH_4Cl, and 5 mM $Mg(OAc)_2$. The 70S fractions were collected and brought to 7 mM β-mercaptoethanol and 10 mM $Mg(OAc)_2$ before concentration by further centrifugation for 24 h at 25 000 rpm in a rotor no. 30 (Spinco centrifuge). The 70S pellet was then rehomogenized in buffer (10 mM Tris-HCl (pH 7.5), 100 mM NH_4Cl, 10 mM $Mg(OAc)_2$, and 7 mM β-mercaptoethanol) and stored frozen at −90 °C. Light-scattering measurements show that 100% of these ribosomes were associated at 5 mM magnesium. They correspond to the A-type ribosomes (Debey et al., 1975) or the "tight" couples described by Noll et al. (1973). Before use each ribosomal stock solution was reactivated by heating for 15 min at 37 °C.

IF-2 initiation factor was prepared from the supernatant of the high-salt ribosome wash according to a previously described procedure (Lelong et al., 1970) but with an additional purification step for IF-2 on a phosphocellulose column (1.5 × 95 cm; elution by NH_4Cl gradient from 0.15 to 0.7 M) before the concentration step on a DEAE-cellulose microcolumn (2.5 × 1.3 cm; eluted with 0.35 M NH_4Cl).

The protein concentration of IF-2 (230 μg/mL) was estimated according to the procedure described by Schaffner & Weissman (1973).

Tests. A. GTPase Activity. Ribosome-dependent IF-2 GTPase activity was measured by the amount of ^{32}P liberated during the reaction according to the procedure of Kolakofsky et al. (1968).

The incubation mixture (25 μL) contained Tris, 50 mM (pH as indicated); 70S ribosomes, 17 pmol (0.70-A_{260} unit), magnesium acetate, 10 mM; ammonium chloride, as indicated; (γ-^{32}P)GTP, 800 pmol (specific activity 410 cpm/pmol); IF-2, 0.23 μg (2.3 pmol) or as indicated; poly(A,U,G), 0.145-A_{260} unit; and ^{3}H-fMet-tRNA, 13.5 pmol. The reaction is stopped after a 10-min incubation at 37 °C by the addition of 0.1 mL of 1 M perchloric acid and 1 mL of 1 mM KH_2Po_4. Under these conditions the reaction is linear as a function of time up to 30 min. After a 10-min centrifugation (10000g) an 0.8-mL aliquot is taken, and 0.5 mL of 5% ammonium molybdate in 4 N H_2SO_4 is added, followed by the addition of 2 mL of a mixture of 2-methylpropanol and benzene (1:1). The tubes were stirred vigorously for 30 s on a vortex, followed by a brief centrifugation to separate the phases. One milliliter of the upper organic phase is counted in 5 mL of toluene, POPOP, PPO/Triton in proportion 2/3:1/3 (v/v).

B. Light-Scattering Measurements. The light-scattering measurements used to study the equilibrium between 30S, 50S, and 70S particles at various pH values were performed on a Jobin Yvon Bearn fluorescence spectrophotometer. Both excitation and emission monochromators were set to 436 nm and the slits were set for a 6-nm band pass (Debey et al., 1975; Wishnia et al., 1975). Measurements were performed in the following medium: sodium cacodylate, 50 mM (pH 6.5), or Tris-HCl, 50 mM (pH 7.6 and 8); NH_4^+ varying between 50 and 100 mM; magnesium acetate varying between 0.5 and 10 mM; and β-mercaptoethanol, 7 mM.

For a typical measurement the 70S (type A) ribosomes were added (5–20 μL) to 1 mL of buffer and the scattered light value was measured. For each experimental curve the concentration of 70S ribosomes was approximately 5-A_{260} units/mL (120 nM 70S ribosomes). After each measurement the final ribosome concentration was checked by absorption at 260 nm and experimental values were corrected for slight variations.

Results

A. Uncoupled GTPase. As has been described previously, IF-2 can hydrolyze GTP in the presence of ribosomes and in the absence of initiator tRNA, mRNA, and other initiation factors (uncoupled GTPase) (Lelong et al., 1970). The rate of hydrolysis is proportional to the concentration of IF-2 up to a saturation level corresponding to a factor/ribosome molarity ratio of about 1:3 (Figure 1). No hydrolysis occurs in the absence of ribosomes.

To investigate the modulation of the GTPase activity we used a low factor concentration, working within the linear portion of the curve shown in Figure 1. The ribosome/factor ratio is about 8, which is close to the presumed ratio in vivo (Grunberg-Manago et al., 1978). Uncoupled IF-2 dependent GTPase activity is strongly dependent on the Mg^{2+} concentration in the medium for the various pH values used. Generally the enzymatic activity increases with Mg^{2+} concentration until it reaches a plateau at about 10 mM Mg^{2+} at every pH value tried (Figure 2). This dependence of GTPase activity on Mg^{2+} concentration cannot be explained by the association effect of the ribosomal subunits by this divalent cation since we verified that the ribosomes used were already totally associated at 5 mM Mg^{2+} in 50 mM NH_4^+.

We also estimated the effect of monovalent cations such as NH_4^+ on the association equilibrium as a function of pH. The

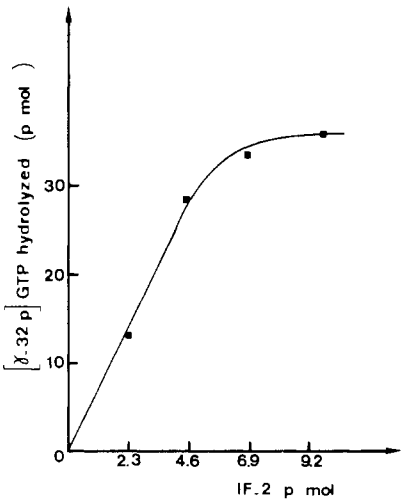

FIGURE 1: Uncoupled GTPase activity of 70S ribosome–IF-2 complex at varying IF-2 concentrations. The incubation mixture (25 μL) contains 50 mM Tris-HCl, pH 7.5; 50 mM NH_4Cl; 10 mM magnesium acetate; 7 mM β-mercaptoethanol; 70S ribosome, 0.70-A_{260} unit (17 pmol); (γ-^{32}P)GTP, 800 pmol; and initiation factor IF-2 (M_r 100 000) as indicated on the abscissa in pmol. Incubation was for 10 min at 37 °C. The intrinsic 70S ribosome hydrolytic activity has been subtracted (1 pmol/10 min) at 10 mM Mg^{2+}.

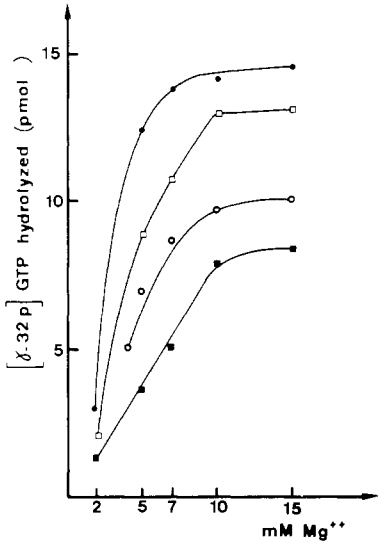

FIGURE 2: Influence of the pH on uncoupled IF-2–GTPase activity as a function of Mg^{2+} concentration. The incubation mixture (25 μL) contains 50 mM Tris-HCl, pH as indicated; 50 mM NH_4Cl; magnesium acetate as indicated; 7 mM β-mercaptoethanol; 70S ribosome, 0.70-A_{260} unit (17 pmol); purified initiation factor IF-2, 2.3 pmol; (γ-^{32}P)GTP, 800 pmol. Incubation was for 10 min at 37 °C. The intrinsic hydrolytic activity of the ribosome has been subtracted. The experiments are at pH 6.5 (■—■); pH 7 (□—□); pH 7.5 (●—●); and pH 8 (○—○).

curve of Figure 3 indicates that for the pH values studied no dissociation can be observed at 100 mM NH_4^+ and 10 mM Mg^{2+}; in the range of ionic strength studied, therefore the pH does not affect the association–dissociation equilibrium. By contrast, for a given ionic strength (Tris, 50 mM; NH_4^+, 50 mM; and Mg^{2+}, 10 mM) the GTPase activity is strongly pH-dependent, an optimum being obtained at pH 7.25 (Figure 4).

There is a strong shift in the pH optimum for IF-2 GTPase activity as a function of ionic strength, as documented in Figure 4 where the GTPase activity is plotted as a function of pH for several values of ionic strength. At low ionic strength (I = 25 mM) the maximal hydrolytic activity occurs at pH 7.5;

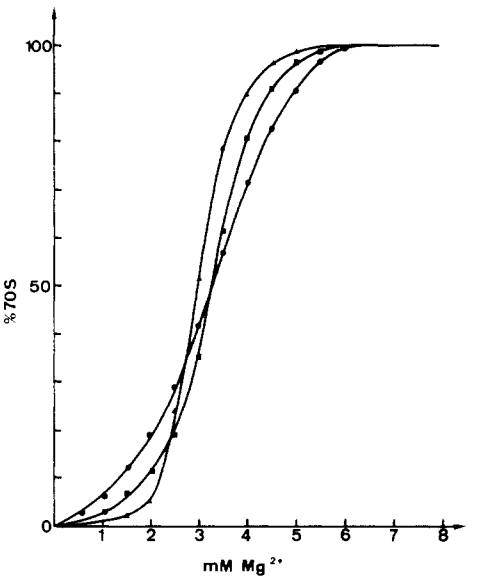

FIGURE 3: Influence of the pH on 70S ribosome dissociation as a function of Mg^{2+} concentration measured by light scattering. The measurements were performed in the buffers as indicated in Materials and Methods at 20 °C and in 100 mM NH_4^+. The 0 and 100% associations were respectively obtained from the light scattering measurement in buffers containing 0.5 and 10 mM Mg^{2+}: pH 6.57 (●—●); pH 7.6 (■—■); pH 8 (▲—▲).

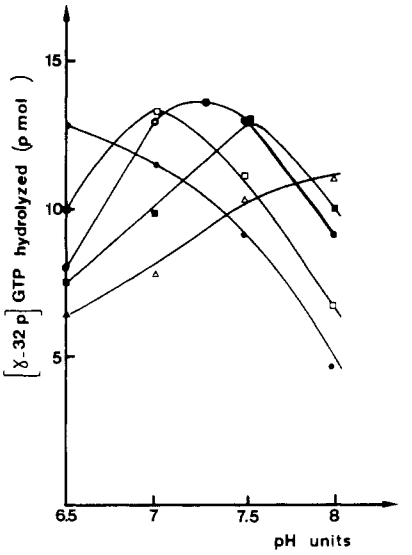

FIGURE 4: Influence of NH_4^+ on the optimal pH for IF-2 uncoupled GTPase activity. The incubation mixture (25 μL) contains 50 mM Tris-HCl, pH as indicated; 10 mM magnesium acetate; NH_4^+ concentration as indicated in the figure; 70S ribosome, 0.70-A_{260} unit (17 pmol); initiation factor IF-2, 2.3 pmol; and (γ-^{32}P)GTP, 800 pmol. Incubation was for 10 min at 37 °C. The intrinsic hydrolytic activity of the 70S ribosome has been subtracted. The experiments are NH_4^+: 15 mM (△—△); 25 mM (■—■); 50 mM (○—○); 75 mM (□—□); and 100 mM (●—●).

when the ionic strength is increased the pH optimum of the reaction is shifted toward more acidic values (7.25, 7.0, and below 6.5 for 50, 75, and 100 mM NH_4^+, respectively). Therefore, the GTPase activity corresponding to the optimum pH is dependent on ionic strength and the shifts in pH activity profiles can exceed two units.

A second way of describing the pH–ionic strength effect is reported in Figure 5 where GTPase activity is plotted as a function of ionic strength for a given pH value. As can be seen, the higher the pH of the sample, the lower the ionic strength ensuring optimal activity; this is a corollary of the results

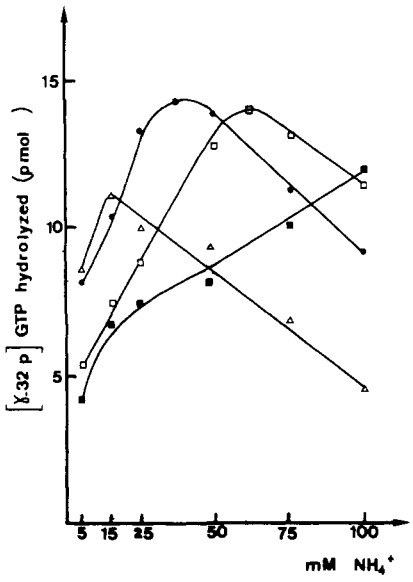

FIGURE 5: Influence of pH on the optimum NH_4^+ concentration on uncoupled IF-2 GTPase activity. Conditions are the same as in Figure 4. The experiments are at pH 6.5 (■—■); pH 7 (□—□); pH 7.5 (●—●); and pH 8 (△—△).

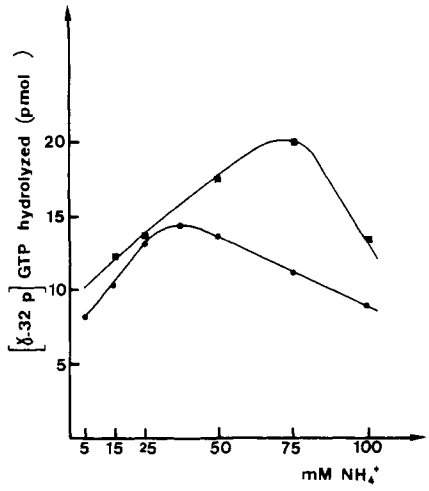

FIGURE 6: Shift of the NH_4^+ optimum of the IF-2 coupled GTPase activity as compared to the uncoupled IF-2 GTPase (pH 7.5). Conditions are the same as in Figure 4 excepted that, for the coupled GTPase activity, the incubation mixture (25 μL) contains in excess of 13.5 pmol of fMet-tRNA^{fMet} and 0.145-A_{260} unit of poly(A,U,G). The experiments are uncoupled (●—●) and coupled (■—■) GTPase activity at pH 7.5.

reported in Figure 4 where the optimal values are displaced toward acidic pH values as ionic strength is increased. The reaction is thus under the tight control of both pH and ionic strength which appear to be narrowly interdependent. These results should be compared to those obtained by Sander et al. (1978) for EF-G GTPase activity modulation by monovalent cation K^+ and pH.

B. Coupled GTPase. When initiator tRNA, fMet-tRNA^{fMet}, and polymer poly(A,U,G) containing the initiator codon AUG are added to the ribosome and IF-2, the hydrolysis of GTP is increased as has been found previously (coupled GTPase; Grunberg-Manago & Gros, 1977).

Figure 6 describes the ionic strength activity profiles obtained at pH 7.5 in the case of the coupled IF-2 GTPase reaction, i.e., in the presence of fMet-tRNA^{fMet} and poly(A,U,G). The curve presents a pattern similar to those obtained for uncoupled reactions (Figure 5), but a shift in the

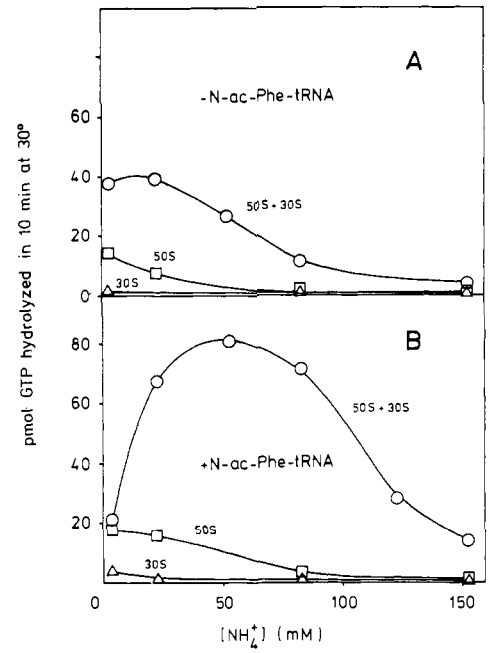

FIGURE 7: Shift by AcPhe-tRNA and poly(U) of the NH_4^+ optimum for the IF-2 GTPase reaction. The reaction mixtures contained, in 75 μL of 50 mM Tris-HCl (pH 7.8 at 20 °C), 10 mM $MgCl_2$, 7 mM β-mercaptoethanol, the indicated concentration of NH_4Cl, 20 pmol of 50S subunits (□), 20 pmol of 30S subunits (△), or 20 pmol each of 50S and 30S subunits (○) prepared as described (Sander et al., 1975), 4 pmol of IF-2, 600 pmol of (γ-^{32}P)GTP, minus (A) or plus (B) 25 pmol of AcPhe-tRNA, and 5 μg of poly(U). After incubation for 10 min at 30 °C, GTPase activity was determined as the amount of $^{32}P_i$ liberated (Sander et al., 1975).

optimal activity must be noted. As can be seen, the concentrations of NH_4^+ yielding optimal activity are 40 mM for the uncoupled and 75 mM for the coupled reaction; this indicates that the presence of fMet-tRNA and poly(A,U,G) decreases the pH at which the reaction actually occurs. Comparing Figures 5 and 6, one should note that the yield of hydrolysis in the presence of polynucleotides is about twice that obtained with the uncoupled reaction at the same pH values. The addition of fMet-tRNA and poly(A,U,G) does not, therefore, introduce a different enzymatic behavior, rather it changes significantly the ionic strength–pH characteristics of the reaction.

A similar pattern is found when the normal initiator tRNA is replaced by AcPhe-tRNA^{Phe} plus poly(U), known to be a functional analogue of the former (Lucas-Lenard & Lipman, 1967), and when individual ribosomal subunits are used instead of "tight" 70S ribosomes (Figure 7). The experiment carried out in the presence of both ribosomal subunits (circles) shows a shift of the NH_4^+ optimum by the AcPhe-tRNA from 20 to 50 mM, thus closely resembling the effect of fMet-tRNA seen in Figure 6. With the 50S subunits alone (squares) IF-2 displayed some GTPase activity at low ionic strength, in analogy to elongation factor G (Sander et al., 1978).

Discussion

The results reported in the present paper, i.e., the shift of the activity–pH profile as ionic strength increases (Figure 4), the effect of the latter depending on the pH value (Figure 5), can usefully be compared to those repeatedly observed with other enzymatic systems bound to polyelectrolyte carriers (Engasser & Horvath, 1975; Goldstein et al., 1964) or in tight electrostatic interaction with soluble and insoluble polyelectrolytes (Byrnes & Downey, 1973; Raae et al., 1975; Maurel & Douzou, 1976). When these enzymes are under such

conditions, their catalytic behavior is markedly modified as compared to normal conditions, that is, when the enzymes are solubilized in the absence of polyelectrolytes. In particular, at low ionic strength the pH–activity profiles of the enzymes bound to polyanions are displaced toward alkaline pH values, as compared to those generated with free enzymes. As the ionic strength is increased these pH profiles revert to their usual positions. Goldstein et al. (1964) and Engasser & Horvath (1975) have shown that these results can satisfactorily be explained in terms of the polyelectrolyte theory.

The microenvironment of the polyelectrolyte on which an enzyme is bound represents a strong electrostatic field developed by the permanent electric charges. Under such conditions the local concentration of any charged molecule or ion is different from its concentration measured in the "bulk" solution. In particular, protons will be more concentrated in the electrostatic field (negative in the case of a polyanion) than in solution.

$$[H^+]_{in} = [H^+]_{out} \exp(-\Sigma\bar{\Psi}/kT) \quad (1)$$

Subscripts in and out refer to the local and bulk phase moieties, respectively, Σ is the unit charge, $\bar{\Psi}$, the electrostatic potential; k, the Boltzman constant, and T, the absolute temperature.

Equation 1 shows that the local protonic concentration in the field developed by a polyanion will be higher than that of the bulk solution, which is the only one directly accessible by experimentation. Therefore pH_{in} will be lower than pH_{out}, the difference, ΔpH, being proportional to the local negative electrostatic potential as shown in eq 2. Similarly any ionizing

$$\Delta pH = pH_{in} - pH_{out} - 0.43\Sigma\bar{\Psi}/kT \quad (2)$$

group under the influence of the potential will have an apparent pK, $pK(\bar{\Psi})$, given by eq 3, where pK_0 is the value of pK in the

$$pK(\bar{\Psi}) = pK_0 - 0.43\Sigma\bar{\Psi}/kT \quad (3)$$

absence of electrostatic potential.

At low ionic strength, this potential ($\bar{\Psi}$) has a maximum, negative value, the apparent pK values (pK_1 and pK_2), determined by the pH profile, become much higher [$pK_1(\bar{\Psi})$, $pK_2(\bar{\Psi})$], and the pH profile is shifted toward alkaline pH values (Figure 4). Increasing ionic strength causes a decrease of the potential, hence a decrease of the pK, and therefore a shift toward acidic pH values (Figure 4). Results reported in Figure 5 are a different representation of the same physical process and confirm the tight interdependence between pH and ionic strength.

Both types of results clearly show that IF-2 dependent GTPase activity can be easily controlled in vitro through protons and cations modulating the electrostatic potential. Many other enzyme-catalyzed reactions occurring in nucleoprotein systems might be controlled in this way (Douzou & Maurel, 1977) as a consequence of the involvement of polyanions represented by polynucleotides. None of these reactions can be satisfactorily analyzed in the classical way (as enzymic processes occurring in an homogeneous medium), but by taking into account the polyanionic nature of their environment this becomes possible.

Since the electrostatic potential $\bar{\Psi}$ value is a steeply decreasing function of the distance between the segment of polyelectrolyte developing this field and the enzyme ($\Sigma\bar{\Psi}/kT$ becomes practically negligible at less than 25 Å for an ionic strength of 0.1 M), the pH–ionic strength dependent behavior of an enzyme implies a rather immediate neighboring with a polyelectrolyte (Kotin & Nagasawa, 1962). This means, in the present case, that the IF-2 catalytic site is under the close influence of a polyanion, presumably ribosomal RNA in the uncoupled GTP hydrolysis, without any interposing proteins which would present a screen between the catalytic site and the RNA, thus reducing the effect of the electrostatic potential.

Comparison of curves in Figure 5 with those in Figures 6 and 7 done at the same pH (7.5) clearly shows that the addition of initiator tRNA and mRNA markedly changes the microenvironment, these polynucleotides bringing about a much more acidic environment (since the higher the ionic strength yielding optimal activity, the lower the pH at which the reaction occurs; see Figure 4). This effect is accompanied by an increase in hydrolysis yield (compare Figure 5 with Figures 6 and 7), a result confirming that the enzymic site is now under the additional influence of initiator tRNA and mRNA.

It is not possible to decide at present whether these polyanions affect directly the electrostatic potential at the GTPase center or change the conformation of the complex such that the contribution of the ribosomal RNA is affected.

We are now in a position to compare the effect of the microenvironment on the different factor-dependent GTPase activities. In this respect it is of interest that all of the factor-associated GTPase activities are well separable from one another in their responses to changes in pH and ionic strength. The initiation factor IF-2 shows the strongest modulation by these parameters (see Results). Elongation factor G also shows an interdependence between pH and ionic strength, albeit to a much lesser extent (Sander et al., 1978), suggesting a less direct involvement of ribosomal RNA in catalytic activity than in the case of IF-2. Curiously, elongation factor Tu does not display any such modulation (Sander & Crechet, 1978), suggesting noninvolvement of ribosomal RNA in this case. Therefore, despite the probable existence of a common ribosomal region for triggering all three GTPase reactions (see the review by Grunberg-Manago et al., 1978), different parts of this region appear to be predominant in each case. This is also illustrated by the different importance of protein L7/L12, which is in the order EF-Tu > EF-G > IF-2 (Kay et al., 1973; Sander et al., 1975).

The effect on GTPase function of components known to be associated with the P site of the ribosome is in agreement with their structural proximity. It is known that not only proteins L7/L12 are implicated in the binding site of IF-2 (Grunberg-Manago et al., 1978) but also protein L11 is implicated in GTPase activities (Highland et al., 1975; Maassen & Möller, 1978). On the other hand it has been found that the chemically reactive AUG mRNA triplet, when it is part of the initiator complex, could be cross-linked to proteins L7/L12 and L11, among others; these would therefore appear to be part or neighbors of the P site (Pongs et al., 1976).

In conclusion, investigation of the GTPase activity and its control by the local electrostatic potential leads to an interesting characterization of the catalytic site that may be seen as complementary to the information obtained from direct structural studies.

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References

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

Brot, N. (1977) in *Molecular Mechanisms of Protein Biosynthesis* (Weissbach, J., & Pestka, S., Eds.) pp 375–411, Academic Press, New York, San Francisco, and London.
Byrnes, J., & Downey, K. (1973) *Biochemistry 12*, 4378–4384.
Chinali, G., Wolf, H., & Parmeggiani, A. (1977) *Eur. J. Biochem. 75*, 55–65.
Debey, P., Hui Bon Hoa, G., Douzou, P., Godefroy-Colburn, T., Graffe, M., & Grunberg-Manago, M. (1975) *Biochemistry 14*, 1553–1558.
Dondon, J., Godefroy-Colburn, T., Graffe, M., & Grunberg-Manago, M. (1974) *FEBS Lett. 45*, 82–87.
Donner, D., Villems, R., Liljas, A., & Kurland, C. G. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3192–3195.
Douzou, P., & Maurel, P. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1013–1015.
Dubnoff, J. S., & Maitra, U. (1972) *J. Biol. Chem. 247*, 2876–2883.
Engasser, J. M., & Horvath, C. (1975) *Biochem. J. 145*, 431–435.
Godefroy-Colburn, T., & Grunberg-Manago, M. (1972) *Enzymes, 3rd Ed. 7*, 533–574.
Goldstein, L., Levin, Y., & Katchalsky, E. (1964) *Biochemistry 3*, 1913–1919.
Grunberg-Manago, M., & Gros, F. (1977) *Prog. Nucleic Acid Res. Mol. Biol. 20*, 209–284.
Grunberg-Manago, M., Dondon, J., & Graffe, M. (1972) *FEBS Lett. 22*, 217–221.
Grunberg-Manago, M., Buckingham, R. H., Cooperman, B., & Hershey, J. W. B. (1978) *Symp. Soc. Gen. Microbiol. 28*, 27–110.
Haenni, A. L., & Chapeville, F. (1966) *Biochim. Biophys. Acta 114*, 135–148.
Highland, J. H., Howard, G. A., Oschner, E., Stöffler, G., Hasenbank, R., & Gordon, J. (1975) *J. Biol. Chem. 250*, 1141–1151.
Kay, A., Sander, G., & Grunberg-Manago, M. (1973) *Biochem. Biophys. Res. Commun. 51*, 979–986.
Kolakofsky, D., Dewey, K. F., Hershey, J. W. B., & Thach, R. E. (1968) *Proc. Natl. Acad. Sci. U.S.A. 61*, 1066–1071.
Kolakofsky, D., Dewey, K. F., & Thach, R. E. (1969) *Nature (London) 223*, 694–697.
Kotin, L., & Nagasawa, M. (1962) *J. Chem. Phys. 36*, 873–879.
Lelong, J. C., Grunberg-Manago, M., Dondon, J., Gros, D., & Gros, F. (1970) *Nature (London) 226*, 505–510.
Lucas-Lenard, J., & Lipmann, F. (1967) *Proc. Natl. Acad. Sci. U.S.A. 57*, 1050–1057.
Maassen, J. A., & Möller, W. (1978) *J. Biol. Chem. 253*, 2777–2783.
Maurel, P., & Douzou, P. (1976) *J. Mol. Biol. 102*, 253–264.
Miller, D. L., & Weissbach, H. (1977) in *Molecular Mechanisms in Protein Biosynthesis* (Weissbach, H., & Pestka, S., Eds.) pp 323–373, Academic Press, New York, San Francisco, and London.
Noll, H., Noll, M., Hapke, B., & Van Diejen, G. (1973) in *Regulation of Transcription and Translation in Eukaryotes, 24th Mosbach Colloquium* (Bautz, E. F. K., Karlson, P., & Kersten, H., Eds.) pp 257–331, Springer-Verlag, Berlin and New York.
Pongs, O., Lanka, E., Bald, R. W., Stöffler, G., Petersen, U., & Grunberg-Manago, M. (1976) *10th Int. Congr. Biochem., Proc., 10th, 1976*, abstracts p 116.
Raae, A., Kleppe, R., & Kleppe, K. (1975) *Eur. J. Biochem. 60*, 437–443.
Sander, G., & Crechet, J. B. (1978) in *Ribosomes and Nucleic Acid Metabolism 3* (Zelinka, J., & Balan, J., Eds.) Publishing House of the Slovak Academy of Sciences, Bratislava (in press).
Sander, G., Marsh, R. C., Voigt, J., & Parmeggiani, A. (1975) *Biochemistry 14*, 1805–1814.
Sander, G., Parlato, G., Crechet, J. B., Nagel, K., & Parmeggiani, A. (1978) *Eur. J. Biochem. 86*, 555–563.
Schaffner, W., & Weissman, C. (1973) *Anal. Biochem. 56*, 502–514.
Stöffler, G., & Wittman, H. G. (1977) in *Molecular Mechanisms of Protein Biosynthesis* (Weissbach, J., & Petska, S., Eds.) pp 117–202, Academic Press, New York, San Francisco, and London.
Voigt, J., Sander, G., Nagel, K., & Parmeggiani, A. (1974) *Biochem. Biophys. Res. Commun. 57*, 1279–1286.
Wishnia, A., Boussert, A., Graffe, M., Dessen, P., & Grunberg-Manago, M. (1975) *J. Mol. Biol. 93*, 499–515.