

ATPase and GTPase Activities Copurifying with GTP-Binding Proteins in *E. coli*

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Abstract

Intrinsic GTPase activity of GTP-binding proteins plays the vital role in regulating the downstream activation pathway. We examined the GTP and ATP hydrolyzing (NTPase) abilities of various bacterial and human GTP-binding proteins under different metabolic conditions. Two metabolic components, acetate and 3-phosphoglyceric acid (3-PG), have shown significant stimulatory action on NTPase activity of G-protein preparations. Acetyl phosphate and 2,3-bisphosphoglyceric acid (2,3-BPG) blocked these stimulations. From gel filtration analyses, we have determined two fractions containing metabolite-inducible NTPase activities which are independent of GTP-binding protein enzymatic actions. Therefore, one should be cautious when NTPase activity is examined in a buffer containing acetate often used for NTPase assay.

GTP-binding proteins are often regarded as the "molecular switches" involved in the regulation of cell growth, differentiation, and vesicular transport among other processes (Bourne *et al.*, 1991; Boguski and McCormick, 1993). It is widely accepted that these proteins exhibit an intrinsic GTPase activity that limits the lifetime of the GTP-bound complex. GTPase-activating proteins (GAP) which are specific for the various members of the GTP-binding protein family accelerate this GTPase activity.

Era, an essential *E. coli* G-protein, was originally identified as a sequence homologue of yeast Ras (Ahn *et al.*, 1986; March *et al.*, 1988). Though it has been shown to be associated with vital cellular functions (Lerner and Inouye, 1991; Britton *et al.*, 1998; Sayed *et al.*, 1999), the function of Era still remains elusive. No factors that modulate Era GTPase in a manner similar to the GAPs have been identified in *E. coli*. During one of our attempts to identify a possible GTPase accelerating factor (like GAP or GTPase activating protein for Ras family proteins) for

E. coli Era GTPase, we observed a high GTPase activity (approximately 1700 units; for simplicity 1 unit is defined as one mmol GTP or ATP hydrolyzed per min per μg of various protein preparations used in this study) in a buffer containing 10 mM potassium acetate in 25 mM Tris-HCl, pH 7.5, 5 mM MgCl_2 , 1 mM DTT and 10 μM [γ -³²P] GTP (NEN Life Science Products). However, when acetate was omitted (or replaced with 10 mM potassium chloride) from the reaction buffer, only a weak GTPase activity (4~10 units) was observed.

Since acetate is a major component in *E. coli* energy metabolism, we consequently have examined all of the other significant components of metabolic pathways, *i.e.*, each intermediates of glycolysis, TCA cycle, 20 amino acids, aminolevulinic acid, porphyrin, FAD, NADH, NADPH, creatine phosphate, riboflavin, pyridoxine ribonucleotides, Ap4A, etc. In addition to the above effect of acetate, only 3-phosphoglyceric acid (3-PG) showed some effect on the stimulation (~3-fold at 10 mM 3-PG) of GTPase activity as determined by the release of free [γ -³²P] Pi. Quite unexpectedly, a high amount of free [γ -³²P] Pi was also observed when [γ -³²P] ATP, instead of [γ -³²P] GTP, was included in the above reaction containing 10 mM acetate or 10 mM 3-PG. In the absence of such metabolic factors, no notable ATPase activity was detected in the Era sample. Furthermore, the induction of NTPase (ATPase and GTPase) was dependent on the concentrations of acetate and 3-PG (Figure 1). While screening for an inhibitor of

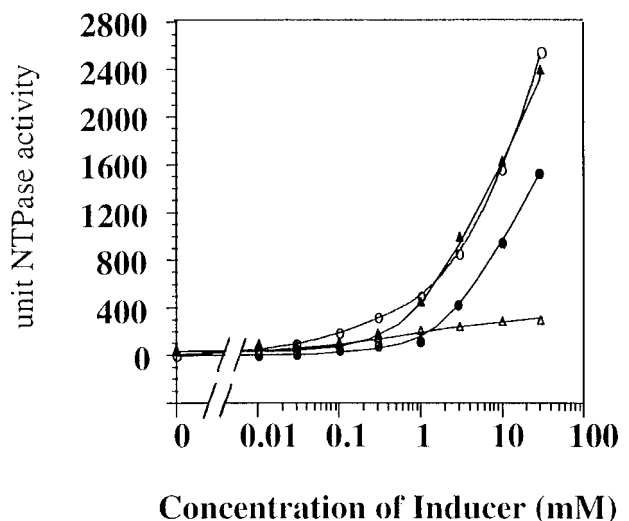


Figure 1. ATPase and GTPase activities of Era preparations measured at different concentrations of potassium acetate or 3-PG. The reactions were performed for 10 min at 37°C as described in the text. The reaction was stopped by the addition of 6% (w/v) activated charcoal, centrifuged at 10,000xg and free [γ -³²P] Pi in the supernatant was measured in a liquid scintillation counter (LKB). Circles represent ATPase while triangles represent GTPase. Open and filled symbols depict NTPase activities in the presence of 3-PG and acetate, respectively.

Received December 13, 1999; accepted January 17, 2000.

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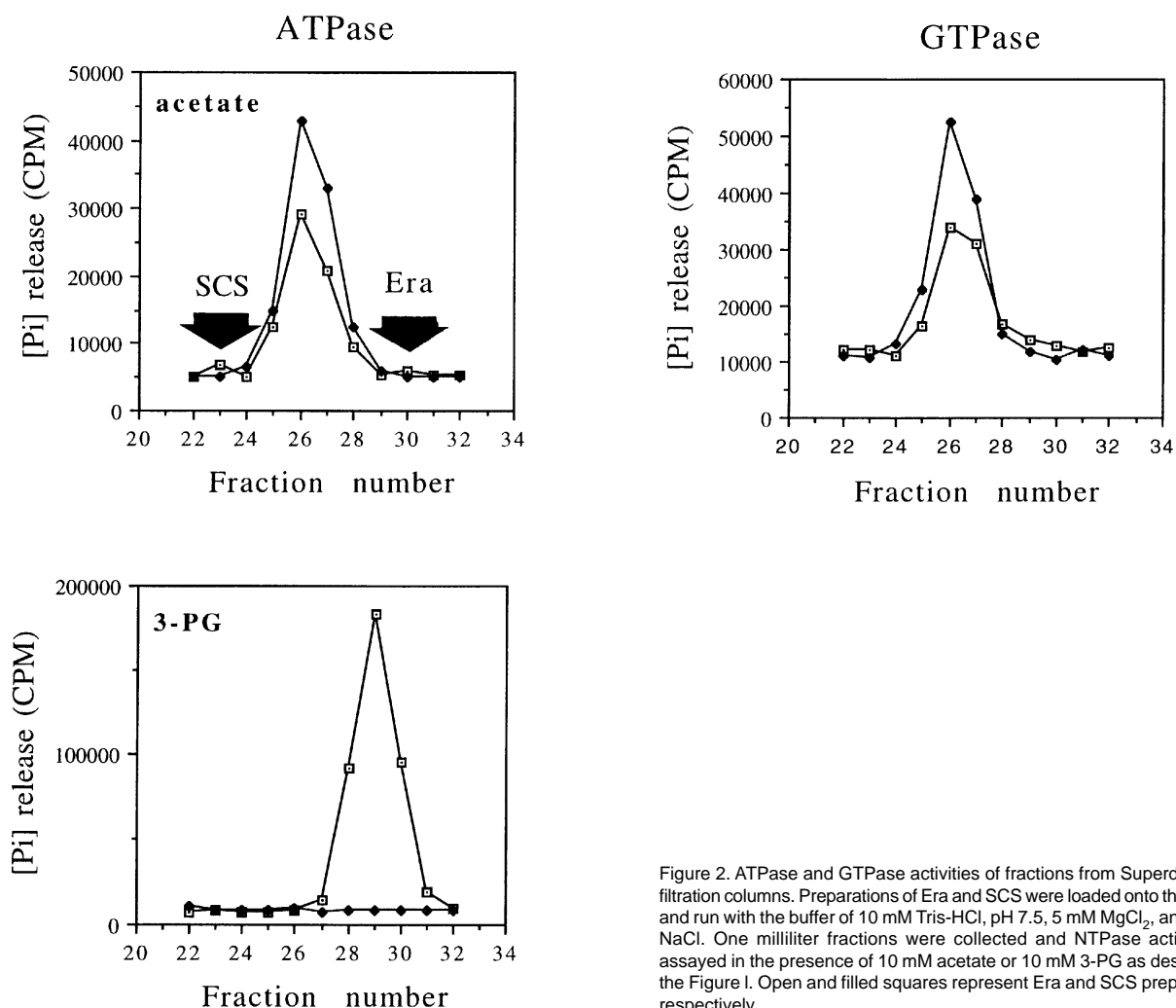


Figure 2. ATPase and GTPase activities of fractions from Superdex G200 filtration columns. Preparations of Era and SCS were loaded onto the column and run with the buffer of 10 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, and 30 mM NaCl. One milliliter fractions were collected and NTPase activity was assayed in the presence of 10 mM acetate or 10 mM 3-PG as described in the Figure 1. Open and filled squares represent Era and SCS preparations, respectively.

these acetate and 3-PG stimulated NTPase, we found that among the various metabolites only acetyl phosphate and 2,3-bisphosphoglyceric acid (2,3-BPG) have inhibitory effects. At 10 mM of concentration, both acetyl phosphate and 2,3-BPG could almost completely nullified the stimulatory action of 10 mM acetate or 10 mM 3-PG. We have also found that this inhibition of acetyl phosphate or of 2,3-BPG was not due to the chelation of Mg²⁺, since the addition of excess Mg²⁺ did not help in blocking the inhibitory effects.

Recently, Brian *et al.* (1999) described that Era binds with *E. coli* succinyl CoA synthetase (SCS). Using anti SCS antiserum (kindly provided by Dr. W. Wolodko, University of Alberta), we have also detected SCS (both α and β subunits) in our Era preparations which have been purified by MonoQ (Pharmacia) ion exchange chromatography as described previously (Sayed *et al.*, 1999). As such, we obviously examined whether our above observation of the acetate and 3-PG effects is due to the SCS or not. We have obtained a highly (Affibule gel) purified SCS (obtained from Dr. W. Wolodko, Alberta University). Expectedly, we have found that an acetate-dependent NTPase was present in the SCS preparation. At 10 mM of potassium acetate unit activities were 1900 and 1100 respectively for

ATPase and GTPase in the SCS preparation. However, unlike that of the Era preparation, no or only a weak induction by 3-PG was observed using SCS preparation. Clearly, it indicated that acetate and 3-PG targets are different and raises the possibility of two enzymatic entities, one (present both in Era and SCS preparations) of which is induced by acetate while the other (present in Era preparation) can be stimulated by both 3-PG and acetate. Thus, we have proceeded to fractionate the acetate and 3-PG inducible NTPase activities from SCS and Era preparations using Superdex G200 (Pharmacia) gel filtration column (Figure 2). The peak elution fractions for *E. coli* SCS (142 kDa) and Era (35 kDa) were 23 and 30, respectively, whereas fractions 26 (corresponds to a molecular mass of ~90 kDa) from both Era and SCS gel filtrations contain the acetate stimulating factor(s), while fraction 29 (corresponds to a molecular mass of ~40 kDa) contains the 3-PG inducible factor(s). The later factor is present exclusively in the Era preparation. It was evident that neither SCS nor Era by itself possessed the observed acetate/3-PG dependent NTP hydrolyzing activities. The acetate-inducible and 3-PG inducible NTPase activities were independent of the presence of Era or SCS.

Recently, Barthel and Walker (1999) have encountered similar problems with the rate of the ATPase activity of DnaK. These authors carefully re-evaluated the ATPase activity of DnaK and have concluded that the previously observed initial burst of ATPase activity was not due to DnaK itself but probably because of the copurified tetramer (66 kDa) of nucleoside diphosphate kinase (NDK). Interestingly, the buffer they used in their study contains a high amount (11 mM) of magnesium acetate. Previously, it was reported that *Pseudomonas aeruginosa* Ras-like protein Pra binds with *E. coli* NDK (Chopade *et al.*, 1997). Consequently, we examined whether NDK contain an acetate or 3-PG dependent ATPase or GTPase, but we could not detect any of such activities with NDK purified from *E. coli* previously (Lu and Inouye, 1996). We have also looked for similar activities of adenylate kinase (ADK) which has been shown to be able to complement NDK function in *E. coli* (Lu and Inouye, 1996). ADK was purified from *E. coli* (*ndk::cmf*) cells as described previously (Lu and Inouye, 1996), and found to contain no acetate or 3-PG dependent ATPase or GTPase.

Finally, we examined two non-*E. coli* GTP-binding proteins expressed in *E. coli*. Obg, an essential protein involved in *Bacillus subtilis* growth and sporulation (Welsh *et al.*, 1994; Vidwan *et al.*, 1995), was obtained as a (His)₆ tagged protein (after Ni²⁺-column affinity purification, a courtesy of Dr. James A. Hoch, Scripps Research Institute, La Jolla, Ca). Obg (His)₆ preparation showed about 4 to 6 fold increase in its intrinsic GTPase activity and a comparable ATPase activities in the presence of both acetate and 3-PG. Human h-Ras, a cell signaling GTPase, was obtained from three independent sources: (a) from a commercial source (Calbiochem), (b) we purified it from an *E. coli* strain overexpressing human h-Ras by MonoQ ion exchange chromatography in a linear gradient of 1 M NaCl in 20 mM Tris-HCl, pH 7.5, 5 mM MgCl₂ and 1 mM DTT after 30% ammonium sulfate precipitation, and (c) gratefully obtained from Dr. Y. Kaziro (Tokyo Institute of Technology). With all of the h-Ras preparations, we could detect very significant ATPase activities (approximately 600 units) only in the presence of 10 mM 3-PG, however not in the presence of 10 mM acetate. The weak intrinsic GTPase activity of h-Ras preparations was found not to be significantly affected by 10 mM 3-PG or 10mM potassium acetate.

All of the proteins described above were obtained from a number of laboratories. All of these proteins were overexpressed in *E. coli*, and had been passed through quite different approaches of ion exchange and affinity based purification steps. The common presence of an inducible high ATPase in these preparations of GTP-binding proteins is curious. It seems that probably two different (3-PG and acetate-sensitive) proteins are present in *E. coli* which have a common feature that they are copurified with GTP-binding proteins during ion exchange chromatography, but could be separated on a Superdex G200 gel filtration column (see Figure 2). At present, we are unable to predict whether these apparent physical interactions of GTP-binding proteins with acetate/3-PG inducible NTPases have any biological relevance, although the stimulatory action of acetate and 3-PG to these unidentified enzymes probably does signify profound metabolic importance. Nevertheless, their identification and appropriate cautions are necessary in defining and

eliminating possible discrepancies regarding the NTPase activities of GTP-binding proteins, at least those purified from *E. coli* expression systems.

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