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Modulation of Glutamine Synthetase Adenylylation and Deadenylylation Is Mediated by Metabolic Transformation of the P_{II}-Regulatory Protein

(*E. coli*/protein-bound uridine nucleotide/adenylyltransferase/2-oxoglutarate/cascade regulation)

M. S. BROWN, A. SEGAL, AND E. R. STADTMAN

Laboratory of Biochemistry, Section on Enzymes, National Heart and Lung Institute, NIH, Bethesda, Maryland 20014

Contributed by E. R. Stadtman, September 15, 1971

ABSTRACT **Earlier studies showed that two protein components, P_I and P_{II}, are concerned with the adenylylation and deadenylylation of *Escherichia coli* glutamine synthetase (EC 6.3.1.2). P_I by itself catalyzes both adenylylation and deadenylylation, but its activity is modulated by the P_{II}-protein and by glutamine, 2-oxoglutarate, ATP, and UTP. The P_{II}-protein exists in two forms: one form, P_{II}-AT, stimulates P_I-catalyzed adenylylation activity in the absence of glutamine and makes this activity very sensitive to inhibition by 2-oxoglutarate; it does not affect deadenylylation activity. The other form, P_{II}-DA, stimulates adenylylation only if glutamine is present, and also stimulates the deadenylylation activity of P_I, which is then dependent upon the presence of ATP and 2-oxoglutarate. Conversion of P_{II}-AT to P_{II}-DA requires the presence of UTP, ATP, and 2-oxoglutarate; it is catalyzed by an enzyme present in P_I preparations. UTP may be directly involved in this conversion since P_{II}-DA fractions reisolated by filtration through Sephadex G-100 contain small quantities of a bound uridine derivative that lacks the γ-phosphoryl group of UTP. The activity of P_{II}-DA, but not of P_{II}-AT, is destroyed by treatment with snake-venom phosphodiesterase. ATP and 2-oxoglutarate apparently function as allosteric effectors for the conversion of P_{II}-AT to P_I-DA.**

A complex enzyme system that catalyzes adenylylation and deadenylylation of *Escherichia coli* glutamine synthetase (EC 6.3.1.2) controls its activity and its response to metabolic effectors (1–7). A protein of molecular weight about 130,000, designated P_I (5), catalyzes both adenylylation (reaction **1**) and deadenylylation (reaction **2**).

glutamine synthetase + ATP →
AMP-synthetase + PP_i (**1**)

AMP-synthetase + Pi → ADP + glutamine synthetase (**2**)

A second protein of molecular weight 50,000 designated P_{II}, stimulates (3, 5) reaction **2**. As originally studied (3, 5, 7), P_{II} had no effect on reaction **1**.

We report here that P_{II} can exist in two forms. One, designated P_{II}-DA, resembles the form previously described (5); it stimulates P_I-catalyzed deadenylylation (reaction **2**) but has little effect on the adenylylation (reaction **1**). The other form, designated P_{II}-AT, markedly stimulates reaction **1**, but has little or no effect on reaction **2**. In the presence of 2-oxoglutarate, UTP, and ATP, an enzyme present in preparations of P_I catalyzes the conversion of P_{II}-AT to P_{II}-DA. Preliminary evidence indicates that this conversion may involve the covalent attachment of a uridine derivative to P_{II}.

Abbreviations: ATase, adenylyltransferase, DA-activity, deadenylylation activity; DTT, dithiothreitol.

MATERIALS AND METHODS

[U-^{14}C]ATP (about 400 Ci/mmol), [U-^{14}C]UTP (350 Ci/mmol), and [^{3}H]ATP (about 10 Ci/mmol) were obtained from New England Nuclear Corp. [γ-^{32}P]UTP (3.5 Ci/mmol) was obtained from International Chemical and Nuclear Corp. The purity of all isotopic compounds was confirmed by thin-layer chromatography. Dithiothreitol (DTT) and 2-oxo glutarate were from Calbiochem. L-glutamine and L-glutamate were from Sigma. All nucleotides were from P-L Biochemicals; Sephadex G-100 was from Pharmacia.

Enzymes. Glutamine synthetase was purified by a zinc precipitation method (R. Miller and E. R. Stadtman, unpublished procedure) from *E. coli* W cells grown on a medium containing 75 mM NH_4Cl and 0.67 M glycerol and harvested after several hours in the stationary phase. This enzyme contained an average of 0.8 equivalent of AMP per mole ($E_{\overline{0.8}}$).

The [^{14}C]adenylylated glutamine synthetase, which served as a substrate for all the deadenylylation experiments, was prepared enzymatically (8) by adenylylation of $E_{\overline{0.8}}$ with [^{14}C]ATP. The glutamine synthetase so prepared contained 11.4 equivalents of AMP per mole ($E_{\overline{11.4}}$), with a specific activity of 20 cpm/pmol of adenylylated subunits.

Partially purified preparations of P_I and P_{II} were obtained (5) from *E. coli* W cells grown on a medium containing 0.67 M glycerol and either 38 or 76 mM NH_4Cl. The cells were harvested about 12 hr after the onset of stationary phase. Purity of the P_I and P_{II}-preparations finally obtained was estimated to be about 40 and 70%, respectively. Both enzymes were stable for several months when stored at −80°C in 20 mM Tris–0.5 mM DTT–0.25 mM $Na_2MgEDTA$ (pH 7.5).

Enzyme Assays. The assay mixture (0.1 ml) for estimation of deadenylylating activity (DA-activity) contained 50 mM 2-methylimidazole·HCl (pH 7.2), 0.02 mM ATP, 1.0 mM UTP, 15 mM 2-oxoglutarate, 20 mM $MgCl_2$, 5 mM Na_2-MgEDTA, 20 mM K phosphate, 1 mM DTT, 100 μg of [^{14}C]ATP-adenylylated glutamine synthetase (equal to 2.0 nmol of adenylylated subunits, 20 × 10^3 cpm/nmol) and enzyme. After 30 min at 37°C, 0.15 ml of 6% $HClO_4$ was added and the mixtures were centrifuged. 0.15 ml of the supernatant was dissolved in 10 ml of Aquasol (New England Nuclear Corp.) and counted in a scintillation spectrometer.

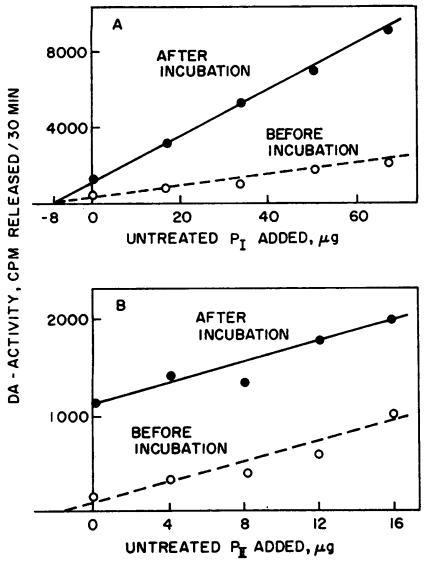

FIG. 1. Activation of P_{II} by incubation with P_I. P_I (150 μg) and P_{II} (35 μg) were incubated together for 60 min at 37°C in 0.2 ml that contained 0.1 mM ATP, 1.0 mM UTP, 5.0 mM 2-oxoglutarate, 1.25 mM $MnCl_2$, 1.0 mM DTT and 50 mM 2-methylimidazole buffer (pH 7.2). At zero time (*open circles*) and after 60 min (*closed circles*) 0.01-ml aliquots, containing 7.5 μg of P_I and 1.75 μg of, P_{II} were removed from the mixture and assayed for deadenylylating activity in the presence of the indicated amounts of nonincubated P_I or P_{II} as shown on the abscissa of *A* and *B*, respectively.

Adenylyltransferase (ATase) activity of P_I-preparations was measured at 37°C in mixtures (0.1 ml) containing 50 mM 2-methylimidazole·HCl (pH 7.2), 20 mM $MgCl_2$, 1 mM DTT, 100 μg of unadenylylated glutamine synthetase ($E_{\overline{0.8}}$)

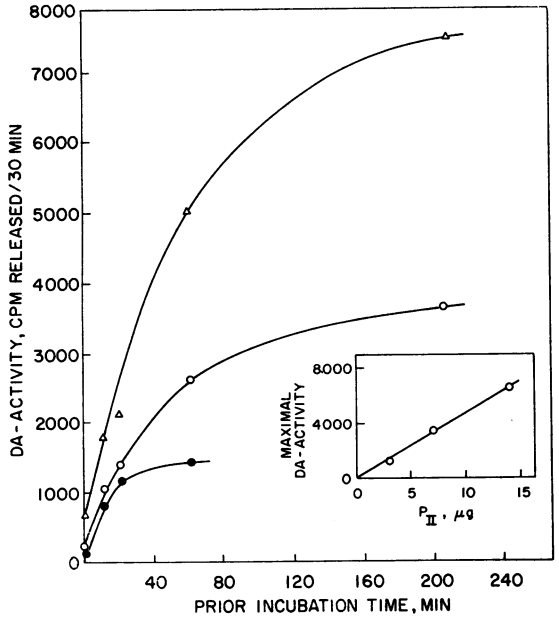

FIG. 2. Effect of P_{II} concentration on rate and extent of DA-activation. 96 μg of P_I was mixed with either 3.5 μg (●——●), 7 μg (○——○), or 14 μg (△——△) of P_{II} in 0.16 ml of solution containing 0.02 mM ATP, 1.0 mM UTP, 15 mM 2-oxoglutarate, 20 mM $MgCl_2$ 1.0 mM DTT, 50 mM 2-methyl-imidazole, and 1 mM $MnCl_2$. At the times indicated on the *abscissa*, 0.02-ml aliquots were removed and assayed for DA activity.

and, where indicated, 1 mM glutamine. After incubation at 37°C, 2 ml of 7% Cl_3CCOOH was added, the tubes were heated at 100°C for 15 min, and the precipitated protein was collected by filtration through Millipore filters of 0.45-μm pore size. The filters were washed with 20 ml of 7% Cl_3CCOOH and 2 ml of ethanol, dried with ether, and counted in a Liquifluor–toluene mixture (New England Nuclear Corp).

RESULTS

Enhancement of the Ability of P_{II} to Stimulate Deadenylylation. As shown in Table 1, prior incubation of P_I with P_{II} in the presence of ATP, UTP, and 2-oxoglutarate led to pronounced enhancement of deadenylylating activity. No activation occurred with prior incubation of P_I or P_{II} separately, either in the absence or in the presence of glutamine synthetase.

Fig. 1*A* shows that after prior incubation with effectors, the DA-activity of a given mixture of P_I and P_{II} is stimulated to a much greater extent by increasing concentrations of P_I. The two curves in Fig. 1*A* are analogous to the results obtained when the concentration of P_I is varied at two different P_{II}-concentrations (3), and suggest that prior incubation leads to an apparent increase in the concentration (i.e., activation) of the P_{II}-component. That P_I is not activated is indicated by the fact that the effective P_I-concentration is not increased by prior incubation. This is evident from the fact that both curves in Fig. 1*A* extrapolate to the same point on the abscissa, which is equal to the negative quantity of P_I present in the mixture before and after prior incubation. The extrapolated value (−8 μg of P_I) agrees with the value of 7.5 μg, which was the amount transferred from the prior incubation mixture. In contrast, Fig. 1*B* shows that the slope of the curves relating P_{II}-concentration and DA-activity is not affected by prior incubation of P_I with P_{II}. Considered together with the data of Fig. 1*A* and the known synergistic

TABLE 1. *Requirement for both P_I and P_{II} in the activation of deadenylylation*

Proteins in prior incubation mixture	Deadenylylating activity pmol/min		Fold activation
	Initially	After prior incubation	
P_I + P_{II}	3.4	18.7	5.5
P_I + P_{II} + synthetase	3.5	18.7	5.4
P_I	3.4	3.8	1.1
P_{II}	4.3	3.9	0.9
P_I + synthetase	3.5	3.1	0.9
P_{II} + synthetase	3.1	3.3	1.1

As indicated, various combinations of 52 μg of P_I, 27 μg of P_{II}, and 36 μg of glutamine synthetase (synthetase; $E_{\overline{8.0}}$) were incubated with 50 mM 2-methyl imidazole (pH 7.2), 0.5 mM ATP, 2 mM UTP, 15 mM 2-oxoglutarate, 1 mM $MnCl_2$, and 1 mM DTT in 0.06 ml at 37°C. Immediately after mixing, and after 60 min, 0.02-ml aliquots were removed and assayed for DA-activity as described in *Methods*. The final assay mixture was supplemented with the protein component omitted from the prior incubation mixture; in all cases, it contained 17.3 μg of P_I and 9 μg of P_{II}.

action of P_I and P_{II} on DA-activity (5), these results contraindicate activation of P_I for deadenylylation and suggest that prior incubation leads to an increase in the ability of P_{II} to stimulate DA activity.

Effect of Time and P_{II}-Concentration on the Activation Reaction. Fig. 2 shows that the enhancement of DA-activity is time dependent, and that both the rate and extent of activation are dependent upon the concentration of P_{II} present during prior incubation. The fact that maximum activity is proportional to the amount of P_{II} added (see insert, Fig. 2) further indicates that prior incubation leads to modification of P_{II}.

Reciprocal Effect of Prior Incubation on DA-Activity and ATase Activity. Data in Fig. 3 show that before its prior incubation, inclusion of P_{II} in the assay mixture caused a 3-fold stimulation of P_I-catalyzed ATase activity and a 2-fold stimulation of DA-activity. Prior incubation with P_I and effectors led to a marked increase in DA-activity and a decrease in ATase activity. This suggests that prior incubation leads to a conversion of P_{II} from a form (P_{II}-AT) that can activate P_I for ATase activity to a form (P_{II}-DA) that activates P_I for DA-activity*.

Isolation of Modified P_{II} from Prior Incubation Mixture. After prior incubation of P_{II} with P_I in the presence of 2-oxoglutarate, ATP, and UTP, the two protein components were reisolated by filtration through a column of Sephadex G-100. As shown in Fig. 4, P_I-ATase and P_{II}-DA stimulatory activities emerged from the column as protein fractions of molecular weight 150,000 and 50,000, respectively. Apparently, prior incubation did not affect the size of either component. Although the fraction of molecular weight 150,000 corresponding to P_I had ATase activity, it contained no DA-activity. Differential loss of DA-activity from P_I-fractions has been observed (ref. 6, and unpublished data) and is attributed to a greater lability of this activity. Both DA-activity and ATase were recovered in the 150,000 molecular-weight fraction in a subsequent experiment in which 2-methylimidazole–$MnCl_2$ buffer was replaced with Tris buffer containing EDTA. Compared to the original P_{II}-preparation, the P_{II}-component reisolated from the prior incubation mixture (fractions 75–95 in Fig. 4) was four times more effective in stimulating DA-activity and only 25% as effective in stimulating ATase activity (in the absence of glutamine). Moreover, further incubation of the reisolated P_{II} with P_I and effectors produced only a slight (1.5-fold) further enhancement of its DA-stimulating activity. These results offer direct proof that prior incubation leads to conversion of P_{II} from a form (P_{II}-AT) that activates ATase activity to a form (P_{II}-DA) that activates DA-activity.

Effector Requirements. Conversion of P_{II}-AT to P_{II}-DA required the presence of 2-oxoglutarate, ATP, and UTP (Table 2). The reaction is strongly inhibited by Pi, which may explain the failure to demonstrate this conversion in earlier studies (3). ADP, AMP, and the β,γ-methylene analogue of ATP were 67, 10, and 50% as effective, respectively, as ATP. Neither GTP nor CTP could replace ATP.

* Inability to detect stimulation of ATase activity by P_{II} in earlier studies (6) was probably due to differences in assay conditions (compare ref. 5).

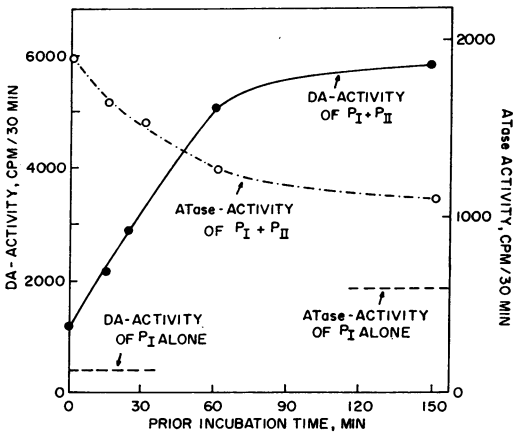

FIG. 3. Reciprocal effect of prior incubation of P_I and P_{II} on ATase and DA-activities. P_I (0.21 mg) and P_{II} (0.10 mg) were incubated together in 0.2 ml of solution containing 0.1 mM ATP, 1.0 mM UTP, 1.0 mM 2-oxoglutarate, 1.0 mM $MnCl_2$, 1.0 mM DTT, and 50 mM 2-methyl-imidazole buffer (pH 7.2). At the indicated times, 0.02-ml aliquots were assayed for ATase and DA-activities under standard assay conditions, except that the ATase assay mixture contained 0.3 mM glutamine, 0.2 mM 2-oxoglutarate, 0.02 mM UTP, and 20 mM K phosphate, in addition to the standard ingredients.

Ability of the β,γ-methylene analogue of ATP to substitute for ATP shows that hydrolysis of the terminal phosphoryl

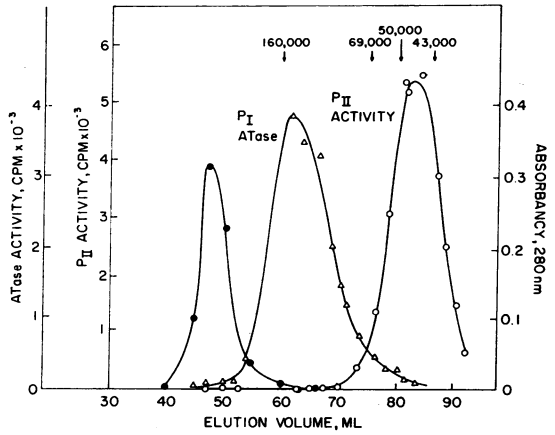

FIG. 4. Isolation of activated P_{II} by gel filtration. 2.55 mg P_I and 0.7 mg P_{II} were incubated at 37°C in 1.5 ml containing 0.1 mM ATP, 1.0 mM DTT, 50 mM 2-methyl-imidiazole buffer 0.2 mM UTP, 15 mM 2-oxoglutarate, and 1.25 mM $MnCl_2$ (pH 7.2). After 3 hr at 37°C, the incubation mixture was applied to a 1.5 × 100 cm column of Sephadex G-100 equilibrated with 50 mM 2-methylimidazole–1.0 mM $MnCl_2$ (pH 7.2). The column had been previously calibrated by measuring the elution volumes of a series of proteins of known molecular weight [Ketose-1-phosphate aldolase (EC 4.1.2.7), 160,000; bovine-serum albumin, 69,000; ovalbumin, 43,000]. 0.06-ml aliquots of each 1.1-ml fraction were assayed for P_I-DA activity by supplementing the standard deadenylylation assay with 10 μg of nonincubated P_{II} and for P_{II}-DA activity (O——O) by supplementing the assay with 43 μg of nonincubated P_I. No P_I-DA activity was detected. ATase activity (Δ——Δ) was estimated as in *Methods*. Fractions (1-ml) 75–91 were pooled, lyophilized, dissolved in 1 ml of water, dialyzed against 20 mM Tris–0.5 mM DTT–0.25 mM MgEDTA, (pH 7.5) buffer, and stored at 4°C. This preparation was used for further study and is referred to hereafter as "P_{II}-DA". ●——●, A_{280}.

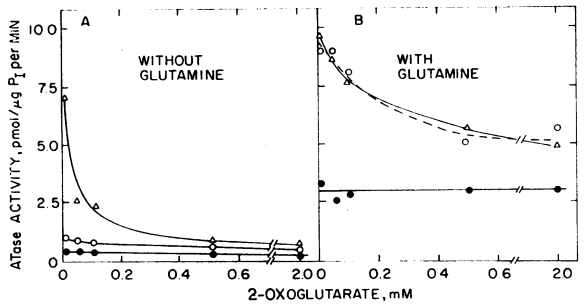

FIG. 5. Effect of P_{II}-DA and P_{II}-AT on adenylylation at different 2-oxoglutarate concentrations, with and without glutamine. ATase assays were performed as described in *Methods*, except that the tubes contained the indicated concentrations of 2-oxoglutarate and, in *A*, no glutamine and, in *B*, 1.0 mM glutamine. Because of the wide range of activities, the amounts of P_I and times of incubation were adjusted so that AMP incorporation remained linear with time. Data are expressed as pmol of AMP incorporated per min per μg of P_I. In *A*, *closed circles* denote P_I alone (42.5 μg for 30 min), *open circles* denote P_I (42.5 μg) plus P_{II}-DA (18 μg) (30-min assay), and *triangles* denote P_I (8.5 μg) plus P_{II}-AT (18 μg) (15-min assay). In *B*, *closed circles* denote P_I alone (8.5 μg for 15 min), *open circles* denote P_I (8.5 μg)plus (18 μg) (15-min assay), and *triangles* denote P_I (8.5 μg) plus P_{II}-AT (18 μg) (15-min assay).

group of ATP is not involved, and indicates that ATP may function as an allosteric effector. UDP is only 4%, and CTP only 20%, as effective as UTP, whereas UDP-glucose is about 75% as affective. Neither pyruvate nor oxalacetate substituted for 2-oxoglutarate.

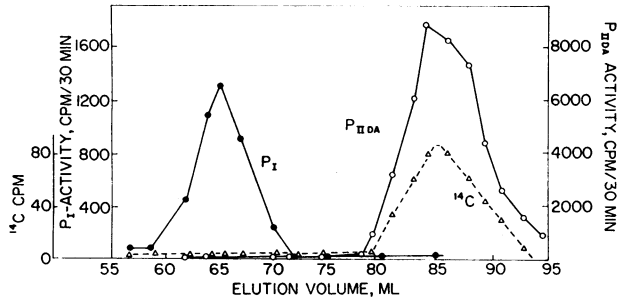

FIG. 6. Association of [^{14}C]uridine with reisolated P_{II}-DA. 1.8 mg of P_{II}-AT was incubated with 0.43 mg of P_I in 1.5 ml containing 50 mM 2-methylimidazole (pH 7.2), 1 mM 2-oxoglutarate, 0.1 mM ATP, 1.0 mM $MnCl_2$, 1.0 mM DTT, and 0.1 mM UTP that consisted of [^{14}C]UTP (53.9 × 10^3 cpm/nmol) and [γ-^{32}P]UTP (15.8 × 10^3 cpm/nmol). After 4 hr at 37°C, the mixture was applied to a 1.5 × 100 cm column of Sephadex G-100 equilibrated with 20 mM Tris–0.5 mM DTT–0.25 mM Na_2Mg-EDTA (pH 7.2) at 4°C, and eluted with the same buffer. 0.05-ml aliquots of each 0.86-ml fraction were dissolved in 10 ml of Aquasol and counted in a liquid scintillation spectrometer. 0.06-ml aliquots were assayed for DA-activity. Assays for P_{II} were supplemented with 21 μg of P_I; the assays for P_I were supplemented with 9 μg of P_{II}-DA. (O——O)=P_I-activity, (●——●) = P_{II}-activity, (Δ——Δ) = cpm of ^{14}C.

The protein concentration in the fraction with peak P_{II}-activity was 0.047 mg/ml by the Lowry method; this is equivalent to 0.94 nmol/ml of a protein of molecular weight 50,000. There were 1420 cpm/ml of ^{14}C; this is equal to 26 pmol/ml of [^{14}C]uridine. Therefore, only one out of 35 protein molecules in this peak was labeled. No ^{32}P counts were detected, even when 0.15-ml aliquots were counted.

TABLE 2. *Effector requirements of the P_{II} conversion reaction*

Prior incubation mixture	Increment in DA-activity pmol/min	Relative activation
Complete	2.40	1.0
−2-oxoglutarate	0.20	0.08
−ATP	0.09	0.04
−UTP	0.06	0.03
Complete + 20 mM K phosphate	0.44	0.18

The complete mixture (0.03 ml) contained 0.02 mM ATP, 1.0 mM UTP, 15 mM 2-oxoglutarate, 20 mM $MgCl_2$, 1.0 mM $MnCl_2$, 50 mM 2-methylimidazole (pH 7.2), 1 mM DTT, 16 μg of P_I, and 2.3 μg of P_{II}. After a 30-min prior incubation at 37°C, the mixtures were assayed for DA-activity. The DA-activity of P_I and P_{II} without prior incubation was 0.5 pmol/min. The data are expressed as the increment in DA-activity due to prior incubation, and as the extent of activation relative to that obtained with the complete mixture.

Effect of Glutamine and 2-oxoglutarate. Earlier studies showed that the DA-activity of relatively impure P_I-preparations is strongly inhibited by glutamine (3), but this inhibitibility is lost with purification or with storage (7). Glutamine did not affect the DA-activity of the (P_I + P_{II})-preparations used in these experiments; however, it was an inhibitor of the P_I-catalyzed conversion of P_{II}-AT to P_{II}-DA. Data in Table 3 show that glutamine inhibition is more marked at high UTP and ATP concentrations than at low.

The ATase activity of P_I is a complex function of P_{II}, 2-oxoglutarate, and glutamine concentrations. As shown in Fig. 5*A*, when glutamine was absent, ATase activity is almost completely dependent on the presence of P_{II}-AT. Little or no stimulation occurred with P_{II}-DA. Moreover, the P_{II}-AT-stimulated activity is very sensitive to inhibition by 2-oxoglutarate; 0.05 mM 2-oxoglutarate inhibited 50%. Data in Fig. 5*B* show that there is significant stimulation of ATase

TABLE 3. *Glutamine inhibition of P_{II} conversion*

Nucleotides in prior incubation mixture (mM)		% Inhibition of P_{II} conversion by 0.4 mM glutamine
UTP	ATP	
1.0	0.1	30
1.0	1.0	82
0.1	0.1	0
0.1	1.0	31

14 μg of P_I and 9.2 μg of P_{II} were incubated together in 0.05 ml of a solution containing the indicated concentrations of nucleotides, 0.5 mM 2-oxoglutarate, and 1.0 mM $MnCl_2$, with and without 0.4 mM glutamine. At 0-time and after 30 min at 37°C, the mixtures were assayed for DA-activity. The data are expressed as the percent inhibition by glutamine of the activation of deadenylylation obtained in the absence of glutamine.

activity by glutamine in the absence of P_{II}; this glutamine-dependent activity is completely insensitive to inhibition by 2-oxoglutarate. In the presence of glutamine, however, further stimulation of ATase activity is produced by either P_{II}-AT or P_{II}-DA. Furthermore, this enhanced activity by either form of P_{II} is inhibited by 2-oxoglutarate.

Possible Role of UTP in the Conversion of P_{II}-AT to P_{II}-DA. Preliminary evidence suggests that the conversion of P_{II}-AT to P_{II}-DA is accompanied by covalent attachment of a uridine derivative to the P_{II}-protein. Fig. 6 shows that when P_I was incubated with P_{II} in the presence of [γ-^{32}P, ^{14}C]UTP and the other effectors of the conversion reaction, the P_{II}-DA that was subsequently isolated by gel filtration contained a significant amount of ^{14}C, but no ^{32}P. In a similar experiment, the P_{II}-DA isolated from prior incubation mixtures containing [^{14}C]UTP and [^{3}H]ATP contained ^{14}C, but not ^{3}H. Treatment of the reisolated, uridine-labeled P_{II}-DA with snake-venom phosphodiesterase (EC 3.1.4.1) led to complete loss of DA-stimulating activity, whereas phosphodiesterase had little effect on the activity of the original P_{II}-AT preparations. Inactivation of P_{II}-DA activity by phosphodiesterase did not restore ATase-stimulating activity.

DISCUSSION

The conversion of P_{II}-AT to P_{II}-DA constitutes another link in the highly sophisticated cascade system of the regulation of *E. coli* glutamine synthetase. This conversion is analogous to the phosphorylation of phosphophorylase kinase by protein kinase in the cascade concerned with the regulation of glycogen metabolism in higher animals (9). Conversion of P_{II} from a form that activates ATase activity to one that stimulates DA-activity facilitates rapid removal of adenylyl groups from glutamine synthetase and, hence, promotes glutamine formation. Since the conversion of P_{II}-AT to P_{II}-DA requires the presence of 2-oxoglutarate and is inhibited by glutamine, it is evident that this conversion is itself subject to metabolite control and constitutes an important site of regulation in nitrogen metabolism. In addition to its role in the transformation of P_{II}, 2-oxoglutarate can directly inhibit adenylylation and stimulate deadenylylation; however, both capacities are dependent on the presence of P_{II} and on the form in which it exists. The P_{II}-protein is thus a vehicle by which the adenylylation–deadenylylation system is sensitized to metabolite control by 2-oxoglutarate. Also, it may be physiologically significant that conversion of P_{II}-DA to P_{II}-AT permits adenylylation to proceed, even in the absence of glutamine, especially if the 2-oxoglutarate concentration is also low. A possible explanation of the UTP requirement for the transformation of P_{II}-AT to P_{II}-DA is afforded by the observation that the uridine moiety of UTP is associated with the P_{II}-DA isolated from prior incubation mixtures. Since the γ-phosphoryl group of UTP is not present, it is possible that the P_{II}-transformation involves the covalent attachment of a UMP or UDP derivative to the P_{II}-protein. The amount of [^{14}C]uridine associated with the reisolated P_{II}-DA corresponds to only 1.0 equivalent per 35 molecules of protein. Incomplete conversion of P_{II}-AT to P_{II}-DA, instability of the uridine–protein bond during reisolation, or impurity of the P_{II}-preparation could account for the poor stoichiometry of uridine binding. Alternatively, the observed binding of the uridine derivative to the P_{II}-DA fraction may be fortuitous and have nothing to do with the P_{II}-transformation. The possibility that the transformation of P_{II} is catalyzed by a contaminating protein in the P_I-preparations, rather than by P_I itself, has not been excluded. If P_I does catalyze the P_{II}-transformation, one must consider the possibility that there is direct interaction of P_{II} with P_I that leads to the displacement of another subunit of molecular weight 50,000, which exhibits DA-stimulating activity.

Whatever the mechanism, if the conversion of P_{II}-AT to P_{II}-DA has a physiological role, then the reverse reaction should also take place. All efforts to demonstrate the conversion of P_{II}-DA to P_{II}-AT have been unsuccessful. Neither P_I nor crude extracts of *E. coli* alone, or in the presence of various effectors, catalyzed the reverse reaction.

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1. Shapiro, B. M., and E. R. Stadtman, *Annu. Rev. Microbiol.*, **24**, 501 (1970).
2. Kingdon, H. S., B. M. Shapiro, and E. R. Stadtman, *Proc. Nat. Acad. Sci. USA*, **58**, 1703 (1967).
3. Shapiro, B. M., *Biochemistry*, **8**, 659 (1969).
4. Wulff, K., D. Mecke, and H. Holzer, *Biochem. Biophys. Res. Commun.*, **28**, 740 (1967).
5. Anderson, W. B., S. B. Hennig, A. Ginsburg, and E. R. Stadtman, *Proc. Nat. Acad. Sci. USA*, **67**, 1417 (1970).
6. Hennig, S. B., W. B. Anderson, and A. Ginsburg, *Proc. Nat. Acad. Sci. USA*, **67**, 1761 (1970).
7. Anderson, W. B., and E. R. Stadtman, *Arch. Biochem. Biophys.*, **143**, 428 (1971).
8. Ginsburg, A., *Methods Enzymol.*, ed. H. Tabor and C. W. Tabor (Academic Press, New York, 1971), Vol. 17, p 927.
9. Stadtman, E. R., *The Enzymes*, ed. P.O. Boyer (Academic Press, New York, 1970), Vol. 1, p. 397.