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Yeast Diadenosine 5′,5‴-P^1,P^4-Tetraphosphate α,β-Phosphorylase Behaves as a Dinucleoside Tetraphosphate Synthetase

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ABSTRACT: The diadenosine 5′,5‴-P^1,P^4-tetraphosphate α,β-phosphorylase (Ap_4A phosphorylase), recently observed in yeast [Guranowski, A., & Blanquet, S. (1985) *J. Biol. Chem. 260*, 3542–3547], is shown to be capable of catalyzing the synthesis of Ap_4A from ATP + ADP, i.e., the reverse reaction of the phosphorolysis of Ap_4A. The synthesis of Ap_4A markedly depends on the presence of a divalent cation (Ca^{2+}, Mn^{2+}, or Mg^{2+}). In vitro, the equilibrium constant $K = ([Ap_4A][P_i])/([ATP][ADP])$ is very sensitive to pH. Ap_4A synthesis is favored at low pH, in agreement with the consumption of one to two protons when ATP + ADP are converted into Ap_4A and phosphate. Optimal activity is found at pH 5.9. At pH 7.0 and in the presence of Ca^{2+}, the V_m for Ap_4A synthesis is 7.4 s^{-1} (37 °C). Ap_4A phosphorylase is, therefore, a valuable candidate for the production of Ap_4A in vivo. Ap_4A phosphorylase is also capable of producing various Np_4N' molecules from NTP and N′DP. The NTP site is specific for purine ribonucleotides (N = A, G), whereas the N′DP site has a broader specificity (N′ = A, C, G, U, dA). This finding suggests that the Gp_4N' nucleotides, as well as the Ap_4N' ones, could occur in yeast cells.

Dinucleoside oligophosphates have been evidenced in numerous organisms [Rapaport & Zamecnik, 1976; Ogilvie & Jakob, 1983; Lüthje & Ogilvie, 1983; Garrison & Barnes, 1984; Morioka & Shimada, 1984; McLennan & Prescott, 1984; Garrison et al., 1986; Segal & Le Pecq, 1986; Baltzinger et al., 1986; reviewed in Silverman and Atherly (1979), Zamecnik (1983), and Grummt (1983)]. Among them, diadenosine tetraphosphate (Ap_4A) has been reported to vary

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with the proliferative state of eukaryotic cells (Rapaport & Zamecnik, 1976; Weinmann-Dorsch et al., 1984a,b; Weinmann-Dorsch & Grummt, 1985; Morioka & Shimada, 1985; Yamaguchi et al., 1985), and it was suggested that this nucleotide might be involved in the initiation of DNA replication [reviewed in Zamecnik (1983) and Grummt (1983)]. However, in the case of prokaryotes, such a role of Ap_4A has been recently disproved (Plateau et al., 1987a).

On the other hand, Ap_4A and the similar adenylylated dinucleoside tri- and tetraphosphates markedly accumulate in response to heat shock or to various oxidative stresses (Lee et al., 1983a,b; Bochner et al., 1984; Denisenko, 1984; Brevet et al., 1985; Baker & Jacobson, 1986; Plateau et al., 1987b; Garrison et al., 1986; Miller & McLennan, 1986). On the basis of this observation, a role of alarmone signaling the onset of the stress was proposed for these nucleotides (Lee et al., 1983a,b; Varshavsky, 1983; Bochner et al., 1984). Finally, Ap_4A and Ap_3A have also been implicated in the modulation of platelet aggregation (Flodgaard & Klenow, 1982; Lüthje & Ogilvie, 1983, 1984).

Because of these ideas, the mechanisms involved in diadenosine tetraphosphate metabolism have been actively studied. Ap_4A can be synthesized in vitro by aminoacyl-tRNA synthetases, through the reaction of ATP with enzyme-bound aminoacyl adenylate (Zamecnik et al., 1966; Plateau et al., 1981; Brevet et al., 1982; Goerlich et al., 1982; Blanquet et al., 1983; Jakubowski, 1983; Led et al., 1983; Rauhut et al., 1985; Wahab & Yang, 1985; Harnett et al., 1985). In the case of some prokaryotic as well as eukaryotic aminoacyl-tRNA synthetases, these reactions are strongly stimulated by the addition of trace amounts of zinc (Plateau et al., 1981; Plateau & Blanquet, 1982; Goerlich et al., 1982; Brevet et al., 1982; Blanquet et al., 1983; Jabukowski, 1983; Goerlich & Holler, 1984; Rauhut et al., 1985). However, the involvement of aminoacyl-tRNA synthetases in the in vivo synthesis of Ap_4A remains to be demonstrated.

The catabolism of dinucleoside polyphosphates seems to be achieved by specific enzymes. Diadenosine tri- or tetraphosphate hydrolases have been characterized in various types of cells [Costas et al., 1985; Lüthje & Ogilvie, 1985; Robinson & Barnes, 1986; Guranowski et al., 1983; Plateau et al. (1985) and references cited therein]. One of them, the *Escherichia coli* Ap_4A hydrolase, has been overexpressed through manipulation of its gene (Mechulam et al., 1985; Blanchin-Roland et al., 1986). The overexpression markedly decreases the Ap_4A concentration in the bacterium, thus demonstrating the involvement of the hydrolase in the Ap_4A metabolism (Mechulam et al., 1985).

In yeast, one catabolic enzyme has been found. This enzyme is particular because it does not hydrolyze Ap_4A but phosphorolyzes it into ATP and ADP (Guranowski & Blanquet, 1985). To be active in vitro, this enzyme requires the presence of divalent metal ions. Among the compounds structurally related to Ap_4A, Ap_5A and Gp_4G are substrates, and ADP, ATP, Ap_4, and Ap_3A are not. In addition, the yeast Ap_4A phosphorylase supports a dinucleoside diphosphate–phosphate exchange reaction (Guranowski & Blanquet, 1986).

In this study, the yeast Ap_4A phosphorylase is shown to ensure the synthesis of Ap_4A from ATP and ADP, i.e., the reverse reaction of the phosphorolytic cleavage of Ap_4A. The metal and pH dependences of this synthetic reaction are investigated. Furthermore, the capability of the enzyme to produce in vitro other dinucleoside tetraphosphates is evidenced. The results raise the possibility that, in yeast, Ap_4A phosphorylase could be an alternative to aminoacyl-tRNA synthetases in the anabolism of dinucleoside tetraphosphates.

Experimental Procedures

Chemicals. Mononucleoside di- and triphosphates and Ap_4A were from Boehringer; Gp_4G was from P-L Biochemicals. Pure samples of Ap_4G, Ap_4C, and Ap_4U were enzymatically prepared with homogeneous *E. coli* lysyl-tRNA synthetase, as already described (Plateau et al., 1985). Alkaline phosphatase from calf intestine (2500 units/mg) and phosphodiesterase from *Crotalus durissus* (1.5 units/mg) were from Boehringer.

Enzyme Purification. Ap_4A phosphorylase from yeast was purified 1700-fold as described (Guranowski & Blanquet, 1985) and stored at −20 °C at a 25 μM concentration (1 mg of protein/mL) in 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer (pH 7.8) containing 50% glycerol and 0.1 mM dithioerythritol. One enzyme unit is the amount of enzyme capable of converting 1 μmol of Ap_4A into ATP + ADP per minute at 37 °C, in a reaction mixture containing 50 mM Tris-HCl (pH 8.0), 120 μM Ap_4A, 5 mM $MgCl_2$, 1 mM potassium phosphate, 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 0.1 mM dithioerythritol.

Phosphorolytic Cleavage of Ap_4A, Ap_4G, and Ap_4C. In a final volume of 50–100 μL, the standard incubation mixture (37 °C) contained 50 mM Tris-HCl (pH 7.8), 20 μg/mL bovine serum albumin (Serva), 0.1 mM dithioerythritol, 0.5 mM $MnCl_2$, 2 mM potassium phosphate, 0.1 mM of the dinucleoside tetraphosphate under study, and catalytic amounts of enzyme (0.003–0.012 unit/mL). The reaction was followed as a function of time by withdrawing aliquots and analyzing both substrates and products with the help of ion-paired high-performance liquid chromatography (HPLC).

Synthesis of Np_4N' (N and N' = A, C, G, U, or dA). In a final volume of 50–100 μL, the standard incubation mixture (37 °C) contained 50 mM Tris-HCl (pH 7.0), 0.1 mM dithioerythritol, 20 μg/mL bovine serum albumin, 0.5–4 mM NTP, 0.5–4 mM N′DP, 0.2–16 mM of the studied metal, and catalytic amounts of enzyme. The reaction was followed as a function of time by freezing aliquots of the incubation mixture and analyzing them later by ion-paired high-performance liquid chromatography (assay A). It was verified that Np_4N' synthesis did not occur in the absence of either N′DP, NTP, or enzyme. Due to a slight contamination of commercial NTPs by Np_4N (0.001–0.01%), a background of Np_4N could be evidenced at time zero of incubation. Data were corrected for this background.

To follow Ap_4A synthesis, another assay was also used (assay B). In this case, aliquots of the incubation mixture were withdrawn, and the reaction was quenched by $HClO_4$ addition [10% (w/w) final concentration]. The sample was then centrifuged, neutralized with 5 M K_2CO_3, and centrifuged again. After dilution (1/3 to 1/20) in 20 mM Tris-HCl (pH 7.8), 1 mM $MgCl_2$, and 0.1 mM $ZnCl_2$, the supernatant was submitted to an alkaline phosphatase treatment (10–50 units/mL, 30 min, 37 °C) in order to ensure the full conversion of the remaining ATP into adenosine, without significant alteration (<5%) of Ap_4A. Ap_4A was then measured by luminescence in a luciferin–luciferase–phosphodiesterase assay, as previously described (Ogilvie, 1981; Brevet et al., 1985).

High Performance Liquid Chromatography. Model 112 pump, Model 210 injector, and Model 160 absorbance detector from Beckman and a Model D2000 Merck chromatointegrator were used. Immediately prior to their analysis by HPLC, the aliquots were made 85% (v/v) by addition of the buffer used for the chromatography. Then, samples of 100 μL were isocratically chromatographed onto a 0.46 × 20 cm column

Table I: Effect of Metal Ions on Initial Rate (s^{-1}) of Ap_4A Synthesis by Yeast Ap_4A Phosphorylase[a]

EDTA	Ca^{2+}	Cd^{2+}	Co^{2+}	Cu^{2+}	Mg^{2+}	Mn^{2+}	Ni^{2+}	Zn^{2+}
0.035	0.88	0.034	0.10	0.015	0.17	0.85	0.015	0.10

[a] Incubation mixture contained 50 mM Tris-HCl (pH 7.0), 4 mM ADP, 4 mM ATP, 0.1 mM dithioerythritol, 20 μg/mL bovine serum albumin, 0.5 mM of the indicated divalent metal ion or 0.1 mM EDTA, and 0.004–0.012 unit/mL enzyme. Initial rates of Ap_4A synthesis were determined by bioluminescence or HPLC analysis, as described under Experimental Procedures.

packed with Lichrosorb RP18 (Merck). The flow rate was 1.5 mL/min, and absorbance of the column effluent was followed at 254 nm.

To resolve the nucleotides in the sample, the following buffers were used: 30 mM potassium phosphate, pH 5.3, 4 mM tetrabutylammonium bromide, and either (i) 13.6% methanol to follow synthesis or phosphorolysis of Ap_4A, Ap_4G, or Gp_4G, (ii) 11.5% methanol (Ap_4U, Ap_4C), (iii) 9% methanol (Gp_4U, Up_4U, Cp_4C, Cp_4U), (iv) 20 mM potassium phosphate, pH 5.3, 4 mM tetrabutylammonium bromide, and 12% methanol (Gp_4C), or (v) 40 mM potassium phosphate, pH 5.3, 5 mM tetrabutylammonium bromide, and 15% methanol (Ap_4dA, dAp_4dA).

Identification of the Np_4N'. Upon incubation of the various NTP and N'DP couples with the enzyme, new nucleotide species (the putative Np_4N' products) could be observed on the chromatogram. In order to establish the nature of these putative Np_4N', they were synthesized in a preparative scale with Ap_4A phosphorylase (in 1-mL reaction mixture, with the buffer conditions of assay A) and further isolated by combination of dihydroxyboryl-Biorex 70 chromatography (Baker & Jacobson, 1984) and of an HPLC step (Lichrosorb RP18 column eluted with 50 mM potassium phosphate, pH 5.3). The identity of the isolated compound was verified by the following criteria: (i) resistance to alkaline phosphatase treatment and (ii) release and identification of the expected nucleoside mono- and triphosphates by limited phosphodiesterase treatment and HPLC analysis.

For instance, the putative Gp_4C product obtained from GTP and CDP was 100% resistant to alkaline phosphatase (5 units/mL, 30 min, 37 °C). After limited phosphodiesterase digestion (0.015 unit/mL, 1 min, 37 °C), it was converted into a mixture of GTP, CTP, GMP, and CMP. In order to precisely determine the concentration of the compound, it was submitted to complete phosphodiesterase hydrolysis, and the resulting NMP and N'MP were quantified by HPLC.

RESULTS

Ca^{2+}, Mn^{2+}, and Mg^{2+} Strongly Stimulate Ap_4A Synthesis. Conversion of a mixture of ATP and ADP into Ap_4A by the yeast Ap_4A phosphorylase was followed at pH 7.0 (37 °C) in the presence of 4 mM ATP and 4 mM ADP, either by HPLC analysis (assay A) or by bioluminescence (assay B). Initial velocities were deduced from linear kinetics, where less than 0.1% of the initial ADP or ATP substrate was transformed into Ap_4A. Velocities obtained by both methods were in close agreement.

The rate of Ap_4A synthesis was rather small (0.035 s^{-1}) in the presence of EDTA. This behavior was not modified by the addition of either Ni^{2+}, Cd^{2+}, or Cu^{2+}. However, as shown in Table I, the reaction rate was greatly enhanced by the addition of metals such as Ca^{2+} or Mn^{2+} and to a lesser extent by that of Mg^{2+}, Zn^{2+}, or Co^{2+}. The optimal concentrations of Ca^{2+}, Mn^{2+}, and Mg^{2+} were determined (Figure 1). The rate of Ap_4A synthesis increases up to 3.0 s^{-1} in the presence of either 3–8 mM Ca^{2+} or 2–4 mM Mn^{2+} and to 1.2 s^{-1} in the presence of 3–8 mM Mg^{2+}. It was verified that the stimulatory effects of the Ca^{2+}, Mn^{2+}, and Mg^{2+} ions were not additive.

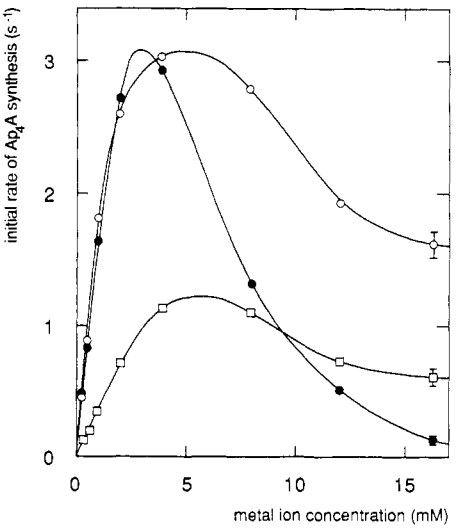

FIGURE 1: Dependence on metal ion concentration of the initial rate of Ap_4A synthesis catalyzed by yeast Ap_4A phosphorylase. The incubation mixture (50–100 μL) buffered with 50 mM Tris-HCl (pH 7.0) contained 4 mM ATP, 4 mM ADP, 20 μg/mL bovine serum albumin, and various $MnCl_2$ (●), $CaCl_2$ (○), or $MgCl_2$ (□) concentrations. After 10 min at 37 °C, Ap_4A concentration was determined by luminescence (see Experimental Procedures).

Michaelis Constants. The initial rate of Ap_4A synthesis was followed as a function of ATP and ADP concentrations (pH 7.0). Ca^{2+} was chosen as the stimulatory metal. Its concentration in the reaction mixture was maintained equal to the sum of the ATP and ADP concentrations.

ATP concentrations ranging from 1 to 40 mM were explored in the presence of fixed 4 mM ADP. In these conditions, the velocity of Ap_4A synthesis followed Michaelian kinetics. A K_m value for ATP equal to 5.7 mM and a V_m equal to 7.4 s^{-1} could be determined.

Similarily, ADP concentrations from 0.1 to 4 mM were explored in the presence of 4 mM ATP. The apparent K_m value for ADP was 0.31 mM.

Equilibrium Constant of the Reaction ATP + ADP $\rightleftharpoons$ Ap_4A + P_i. To determine the equilibrium constant of the reaction [$K = ([Ap_4A][P_i])/([ATP][ADP])$], ATP and ADP (5 mM each) were incubated at 37 °C during 15 min in the presence of 4 mM $CaCl_2$, 0.04–0.2 unit/mL Ap_4A phosphorylase, and 10 mM potassium phosphate. In these conditions, contaminating potassium phosphate brought by ATP and ADP (0.3 mM) was negligible. To ensure that equilibrium was reached at the end of the incubation, it was verified that the addition of 100 μM Ap_4A prior to the incubation did not modify the value of the equilibrium $([Ap_4A][P_i])/([ATP][ADP])$ ratio. At pH 7.0, the K value was equal to 1.6×10^{-3}. As shown in Figure 2, the K value strongly depends on pH. Ap_4A is favored at low pH, a conclusion in agreement with the consumption of one to two protons when ATP + ADP are converted into Ap_4A + P_i.

The effect of pH can be shown also at the level of the rates of Ap_4A phosphorolysis or synthesis. Whereas Ap_4A phosphorolysis is optimal at pH 8.0 (Guranowski & Blanquet, 1985), the highest rate of Ap_4A synthesis is observed at pH

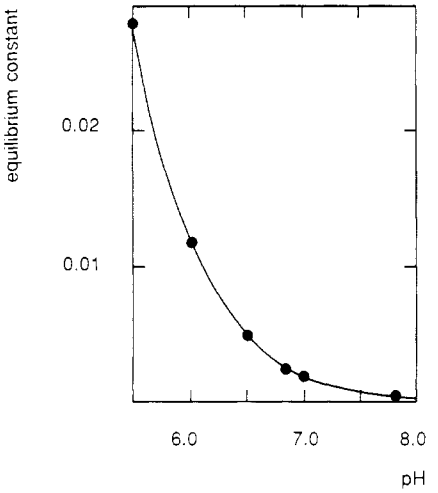

FIGURE 2: Determination of equilibrium constant of the reaction at various pHs. pH values ranging from 5.5 to 8 were obtained with a 50 mM ammonium acetate buffer. The reaction mixture contained 0.1 mM dithioerythritol, 20 μg/mL bovine serum albumin, 4 mM Ca^{2+}, 5 mM ATP, 5 mM ADP, and 10 mM potassium phosphate. After 15 min at 37 °C, Ap_4A concentration was determined by luminescence as described under Experimental Procedures. In order to verify that the equilibrium was actually reached, different enzyme concentrations were used. Moreover, two sets of experiments were performed with or without the addition of 100 μM Ap_4A.

Table II: Effect of pH on Initial Rate of Ap_4A Synthesis Catalyzed by Yeast Ap_4A Phosphorylase in the Presence of Different Metal Ions[a]

pH	initial rate of synthesis (s^{-1})		
	Ca^{2+}	Mg^{2+}	Mn^{2+}
4.8	0.75	0.42	0.41
5.9	4.6	2.0	3.9
7.0	3.3	1.1	2.9

[a] The reaction mixture contained 50 mM ammonium acetate (pH 4.8, 5.9, or 7.0), 4 mM ATP, 4 mM ADP, 0.1 mM dithioerythritol, 20 μg/mL bovine serum albumin, 4 mM $CaCl_2$, $MgCl_2$, or $MnCl_2$, and 0.004–0.012 unit/mL enzyme. Initial rates of Ap_4A synthesis were determined by bioluminescence or HPLC analysis.

5.9, whatever the added metal ion, Mn^{2+}, Mg^{2+}, or Ca^{2+} (Table II). A V value of 4.6 s^{-1} is obtained in the presence of 4 mM Ca^{2+}. This rate is greater than that of Ap_4A phosphorolysis under identical pH and Ca^{2+} conditions (3 s^{-1}, in the presence of 1 mM potassium phosphate and 120 μM Ap_4A).

Yeast Ap_4A Phosphorylase Generates Various Np_4N' (N and N' = A, C, G, U, or dA). Because Ap_4A phosphorylase is capable of phosphorolyzing Gp_4G into GDP + GTP (Guranowski & Blanquet, 1985), Gp_4G synthesis from GDP and GTP was also expected. More generally, the reaction between N'TP and NDP can theoretically lead to Np_4N' formation. Therefore, the evolution of an incubation mixture containing 0.5 mM NTP, 0.5 mM N'DP, 0.2 mM $MnCl_2$, and catalytic amounts of enzyme was systematically followed. At various times (5–30 min), aliquots of the incubation mixture were analyzed by ion-paired HPLC, and the products were assigned as described under Experimental Procedures. In this set of reactions, manganese was used rather than calcium for the sake of comparison with the studies concerning the phosphorolytic properties of the enzyme (Guranowski & Blanquet, 1985).

Initial rates of Np_4N' synthesis from NTP and N'DP are summarized in Table III. The rate strongly depends on the nature of the NTP substrate. With ATP, rates of Ap_4N' synthesis were high and rather constant, whatever the used

Table III: Initial Rates of Np_4N' Synthesis from NTP and N'DP (N and N' = A, C, G, U, or dA)[a]

NTP substrate	N'DP substrate	initial rate of synthesis (s^{-1})	product
ATP	ADP	0.36	Ap_4A
	CDP	0.26	Ap_4C
	GDP	0.52	Ap_4G
	UDP	0.69	Ap_4U
CTP	ADP, CDP, GDP, UDP	<0.010	not observed
GTP	ADP	0.036	Ap_4G
	CDP	0.015	Gp_4C
	GDP	0.12	Gp_4G
	UDP	0.010	Gp_4U
UTP	ADP, CDP, GDP, UDP	<0.010	not observed
dATP	ADP, dADP	<0.010	not observed
ATP	dADP	0.17	Ap_4dA

[a] The reaction mixture contained 50 mM Tris-HCl (pH 7.0), 0.1 mM dithioerythritol, 20 μg/mL bovine serum albumin, 0.2 mM $MnCl_2$, 0.5 mM NTP, 0.5 mM N'DP, and 0.004–0.12 unit/mL enzyme. Initial rates of Np_4N' synthesis were derived from HPLC analysis.

Table IV: Phosphorolysis of Ap_4C and Ap_4G by Yeast Ap_4A Phosphorylase[a]

substrate	products	initial rate of phosphorolysis (s^{-1})
Ap_4C	ATP + CDP	7.5
	ADP + CTP	<0.05
Ap_4G	ATP + GDP	34.6
	ADP + GTP	4.7

[a] The reaction mixture contained 50 mM Tris-HCl (pH 7.8), 0.1 mM dithioerythritol, 20 μg/mL bovine serum albumin, 0.5 mM $MnCl_2$, 0.1 mM Ap_4C or Ap_4G, 2 mM potassium phosphate, and 0.003–0.012 unit/mL enzyme. The initial rates of phosphorolysis were obtained by HPLC analysis.

N'DP (0.26–0.69 s^{-1}). The syntheses from GTP were systematically slower than those from ATP, and the nature of the N'DP substrate became critical. For instance, the rate of Gp_4G synthesis is 0.12 s^{-1}, while those of Gp_4A, Gp_4C, and Gp_4U synthesis are only 0.036, 0.015, and 0.010 s^{-1}, respectively. Syntheses from CTP or from UTP could not be evidenced under our experimental conditions (<0.01 s^{-1}) even when either Ca^{2+} or Mg^{2+} was used instead of Mn^{2+} in the assay. In agreement with the specificity of the phosphorolysis reaction (Guranowski & Blanquet, 1985), Np_3N' synthesis could not be observed, when an NTP + N'DP mixture or N'DP alone was used (<0.01 s^{-1}).

In order to determine the role of the sugar moiety in the recognition of the nucleotides by the enzyme, synthesis of dinucleoside tetraphosphates was undertaken with dATP or dADP as substrates. With ATP + dADP, Ap_4dA synthesis occurred at a rate (0.17 s^{-1}) similar to that of Ap_4A synthesis (0.36 s^{-1}). On the other hand, when dATP + ADP or dATP + dADP were mixed, dinucleoside tetraphosphate synthesis could not be detected (rate of synthesis <0.01 s^{-1}). This indicates that a deoxyribonucleotide can be used in the NDP site, not in the NTP one.

Asymmetry of the Np_4N' Site. The above experiments indicated an asymmetry of the enzymatic site. For example, synthesis of Ap_4C is at least 25 times faster from ATP + CDP than from ADP + CTP (Table III). Such an asymmetry was therefore expected to also occur at the level of the phosphorolysis of Ap_4C and Ap_4G by the enzyme.

As shown in Table IV, upon phosphorolysis, Ap_4C exclusively produces ATP + CDP, not ADP + CTP. Similarily, Ap_4G phosphorolysis gives ATP + GDP at a rate (34.6 s^{-1})

much higher than that for ADP + GTP appearance (4.7 s^{-1}). In the phosphorolytic reaction, the binding of the adenosine moiety is therefore favored in the NTP site of the enzyme. This is well in agreement with the features of the synthetic reaction.

Yeast Ap_4A Phosphorylase Catalyzes an Exchange between the γ-Phosphates of ATP and GTP. The above properties raised the possibility to convert GDP + ATP into ADP + GTP, or vice versa, via Ap_4G synthesis followed by phosphorolysis:

$$ATP + GDP \rightleftharpoons Ap_4G + P_i \rightleftharpoons ADP + GTP$$

Consequently, nucleotides were followed in a medium containing 50 mM Tris-HCl (pH 7.0), 0.1 mM dithioerythritol, 20 μg/mL bovine serum albumin, 2 mM $MnCl_2$, 0.5 unit/mL of yeast Ap_4A phosphorylase, and either 0.5 mM ADP + 0.5 mM GTP or 0.5 mM ATP + 0.5 mM GDP. In either case, ATP + GDP or ADP + GTP appeared linearily with time, at a rate of 0.04 s^{-1}. The absence of lag in the triggering of the reaction suggests that, in the reaction mixture, Ap_4G rapidly reaches its steady-state concentration. On the contrary, ATP could not be formed with ADP + UTP or ADP + CTP as substrate combinations, thus confirming the lack of reaction of CTP or UTP with N′DP to give Cp_4N' or Up_4N'.

Discussion

The yeast Ap_4A phosphorylase was previously characterized through its ability to convert Ap_4A into ADP + ATP (Guranowski & Blanquet, 1985). In this paper, the reverse reaction, i.e., the synthesis of Ap_4A from ADP + ATP, is demonstrated. Since the measured rates of Ap_4A synthesis are similar to the rates of phosphorolysis under identical ion and pH conditions, the question can be asked for whether the yeast Ap_4A phosphorylase actually degrades or synthesizes Ap_4A in vivo.

In yeast cells, total ATP concentration is close to 1.5 mM (Kudrna & Edlin, 1975; Fonzi et al., 1979), that of ADP is 0.35 mM (Fonzi et al., 1979), and that of phosphate is around 7 mM (den Hollander et al., 1981). Recently, Garrison and Barnes (1984) found in yeast 3.6 pmol of Ap_4A/mg of protein, which corresponds to about 0.3 μM cellular Ap_4A. With these values, a ratio $K = ([Ap_4A][P_i])/([ATP][ADP])$ of 4×10^{-3} can be calculated. On the other hand, the intracellular pH of aerobically grown yeast cells is 7.2 (den Hollander et al., 1981). In vitro, at this pH and in the presence of 4 mM Ca^{2+}, we find an equilibrium constant value equal to 1.2×10^{-3}. The similarity between the two K values argues therefore that in vivo the balance between the Ap_4A, ATP, ADP, and P_i pools might reflect the activity of the Ap_4A phosphorylase.

It is remarkable that, in vitro, the synthesis of Ap_4A by the yeast phosphorylase is favored by H^+ and/or Ca^{2+}. Noteworthy, heat shock has been reported to increase the cytosolic concentration of these ions in yeast (Weitzel et al., 1985) as well as in *Drosophila* cells (Drummond et al., 1986). Since heat shock precisely provokes a dinucleoside polyphosphate accumulation in yeast and *Drosophila* cells (Denisenko, 1984; Brevet et al., 1985; Baltzinger et al., 1986), it is tempting to relate the metabolism of Ap_4A to such cellular ionic changes.

However, a causal role of Ap_4A in the induction of heat shock proteins is presently doubtful. In particular, experimental conditions have been found that promote heat shock protein synthesis without any increase in the cellular Ap_4N concentration (Brevet et al., 1985; Guedon et al., 1985; Bloom et al., 1986; Miller & McLennan, 1986). In the context of the above idea, it should be noted that, in *Drosophila* cells, the changes in intracellular pH and calcium associated to heat shock have been recently shown not to be the prerequisite for the induction of heat shock proteins (Drummond et al., 1986).

The enzyme(s) responsible for Ap_4N synthesis in vivo has (have) not yet been identified. Until this work, aminoacyl-tRNA synthetases were the only candidate. Here, it is shown that Ap_4A phosphorylase can sustain Ap_4A synthesis at a maximal rate (V_m = 7.4 s^{-1}) greater than that ensured by a yeast aminoacyl-tRNA synthetase in optimal zinc conditions (≈3 s^{-1}) (Blanquet et al., 1983). Nevertheless, the aminoacyl-tRNA synthetases still are good candidates to account for the synthesis of the Ap_3N nucleotides. Such nucleotides have been found in all examined cells (Lee et al., 1983a,b; Ogilvie & Jakob, 1983; Bochner et al., 1984; Denisenko, 1984; Brevet et al., 1985; Miller & McLennan, 1986).

Another important feature is the capability of yeast Ap_4A phosphorylase to generate various Np_4N' (N and N′ = A, C, G, U, or dA) from NTP and N′DP. One of the enzyme subsites reacts with a trinucleotide with the specificity ATP > GTP ≫ UTP, CTP, and dATP whereas the other subsite reacts with a dinucleotide without marked specificity. The synthesis of Np_4N' from GTP as well as from ATP raises the possibility that Ap_4A phosphorylase functions as a GDP kinase and plays a role in the cellular GTP/GDP balance.

Finally, the capability of the Ap_4A phosphorylase to produce Np_4N' without ATP as indispensable substrate, while aminoacyl-tRNA synthetases cannot, may help to distinguish between the roles of these enzymes in vivo. The occurrence of Gp_4N' (N′ = C, G, or U) in yeast extracts would be a strong indication that the phosphorylase actually behaves in vivo as an Np_4N' synthetase.

Registry No. Ap_4A, 5542-28-9; Gp_4G, 4130-19-2; ADP, 58-64-0; ATP, 56-65-5; Ap_4G, 10527-46-5; Ap_4C, 83008-69-9; GTP, 86-01-1; CDP, 63-38-7; GDP, 146-91-8; UDP, 58-98-0; dADP, 2793-06-8; Ap_4U, 10527-48-7; Gp_4C, 79695-24-2; Gp_4U, 79695-25-3; Ap_4dA, 30632-09-8; Ca, 7440-70-2; Mn, 7439-96-5; Mg, 7439-95-4; Zn, 7440-66-6; Co, 7440-48-4; Ap_4A phosphorylase, 96697-71-1; dinucleoside tetraphosphate synthetase, 108918-79-2.

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