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Regulatory Role of Adenine Nucleotides in the Biosynthesis of Guanosine 5'-Monophosphate

H. J. J. NIJKAMP
Laboratory of Microbiology, State University, Utrecht, The Netherlands

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Derepression of the synthesis of inosine 5'-monophosphate (IMP) dehydrogenase and of xanthosine 5'-monophosphate (XMP) aminase in *pur* mutants of *Escherichia coli* which are blocked in the biosynthesis of adenine nucleotides and guanine nucleotides differs in two ways from derepression in *pur* mutants blocked exclusively in the biosynthesis of guanine nucleotides. (i) The maximal derepression is lower, and (ii) a sharp decrease in the specific activities of AMP dehydrogenase and XMP aminase occurs, following maximal derepression. From the in vivo and in vitro experiments described, it is shown that the lack of adenine nucleotides in derepressed *pur* mutants blocked in the biosynthesis of adenine and guanine nucleotides is responsible for these two phenomena. The adenine nucleotides are shown to play an important regulatory role in the biosynthesis of guanosine 5'-monophosphate (GMP). (i) They induce the syntheses of IMP dehydrogenase and XMP aminase. (The mechanism of induction may involve the expression of the *gua* operon.) (ii) They appear to have an activating function in IMP dehydrogenase and XMP aminase activity. The physiological importance of these regulatory characteristics of adenine nucleotides in the biosynthesis of GMP is discussed.

In *Escherichia coli*, *guaA* and *guaB* are the structural genes of one operon (11). Xanthosine 5'-monophosphate (XMP) aminase [XMP: ammonia ligase (adenosine diphosphate, ADP), EC 6.3.4.1] and inosine 5'-monophosphate (IMP) dehydrogenase (IMP: NAD^+ oxidoreductase, EC 1.2.1.14), the products of these genes, are coordinately under the control of guanosine 5'-monophosphate (GMP) or one of its derivatives (10).

In *guaA* and *guaB* mutants, grown in media supplemented with suboptimal concentrations of guanine, derepression of the syntheses of IMP dehydrogenase and XMP dehydrogenase, respectively, occurs when the cells have exhausted their guanine supply. After the increase in specific activity of these enzymes in the derepression phase, a plateau is reached. Under the same conditions of derepression in a *purH* mutant, which has a biochemical block before the metabolic branch point IMP in purine biosynthesis (*see* Fig. 1), there is a shorter period of increase in enzyme activity, followed by a sharp decrease to a very low level (10). This is in contrast with the course of derepression in *gua* mutants blocked in the pathway leading from IMP to GMP.

This communication describes experiments concerning the differences between the pattern of derepression in the syntheses of XMP aminase and IMP dehydrogenase in *pur* mutants, blocked in GMP synthesis (*guaA* and *guaB*), and the pattern of derepression of these enzymes in *pur* mutants blocked in GMP and AMP syntheses (e.g. *purH*).

MATERIALS AND METHODS

The bacterial strains employed were all derivatives of *E. coli* K-12. The characteristics and origins of the strains are presented in Table 1. The pathways and biochemical blocks in purine biosynthesis relevant to this paper are indicated in Fig. 1.

Culture media for the preparation of cell-free extracts have been described by Magasanik, Moyed, and Gehring (6). Other media used were described by Nijkamp and DeHaan (10).

Bacteria were grown overnight and then diluted 1 to 10 in fresh prewarmed medium. To repress IMP dehydrogenase and XMP aminase synthesis, guanine was added in the final concentrations of 25 μg/ml (overnight culture) or 12.5 μg/ml (log phase culture). When exponential growth was obtained, the bacterial culture was again diluted to 1 to 10 with fresh prewarmed medium. The cultures were grown aerobically in a mechanical shaker at 37 C. Bacterial growth was monitored by measuring the optical density in a Unicam SP 600 spectrophotometer at 660 nm.

For the preparation of cell-free extracts, the cells were rapidly chilled on ice and collected by centrifuga-

TABLE 1. *Characteristics and origin of bacterial strains*[a]

Strain	Mating type	Purine markers[b]	Deficiency	Origin or reference
H 718	F⁻	*guaA*	XMP aminase	Ref. 13
H 712	F⁻	*guaB*	IMP dehydrogenase	Ref. 13
H 677	F⁻	*purC*	SAICAR synthetase	Ref. 13
H 725	F⁻	*purCguaA*	SAICAR synthetase, XMP aminase	Ref. 2
H 724	F⁻	*purCguaB*	SAICAR synthetase, IMP dehydrogenase	Ref. 2
H 680	F⁻	*purB*	Adenylosuccinase	Ref. 13
H 675	F⁻	*purH*	AICAR transformylase	Ref. 13
H 687	F⁻	*purA*	SAMP synthetase	Ref. 13
H 883	F⁻	*purF*	Phosphoribosyl pyrophosphate amidotransferase	Ref. 13
N 27	F⁻	*purAguaB*	SAMP synthetase, XMP aminase	H 839 × H 687
H 839	Hfr	*guaB*	IMP dehydrogenase	Ref. 13

[a] The genetic symbols and nomenclature used are described in Demerec et al. (1) and in Taylor and Trotter (14).

[b] Additional markers for all strains except H 839 were *thi*⁻, *his*⁻, *tyr*⁻, *trp*⁻, *lac*⁻, *gal*⁻, *str*ʳ. For 839, additional markers were *thi*⁻, *leu*⁻, *thr*⁻, *tyr*⁻.

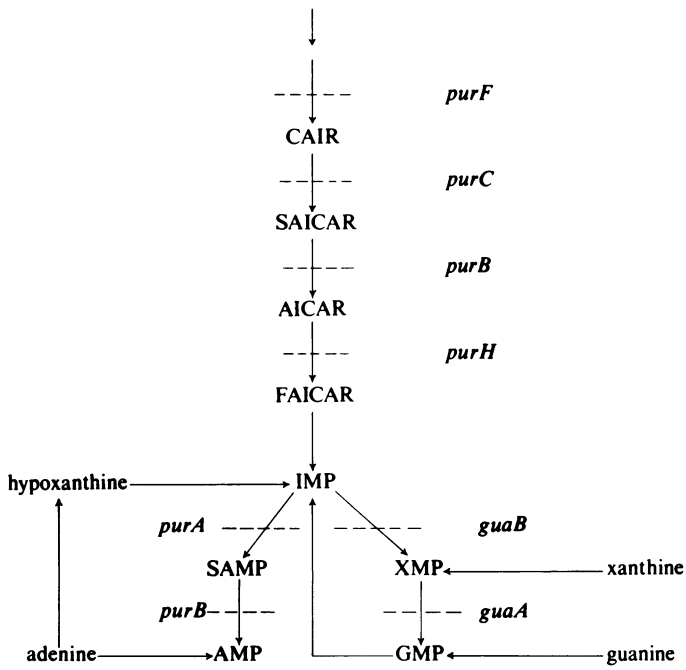

FIG. 1. *Pathway of purine biosynthesis. Abbreviations: CAIR, 5-amino-4-imidazolecarboxylic acid ribonucleotide; SAICAR, 5-amino-4-imidazole-N-succinocarboxamide ribonucleotide; AICAR, 5-amino-4-imidazolecarboxamide ribonucleotide; FAICAR, 5-formamido-4-imidazolecarboxamide ribonucleotide; IMP, inosine 5-monophosphate; XMP, xanthosine 5'-monophosphate; GMP, guanosine 5'-monophosphate; SAMP, succinyl adenosine 5-monophosphate; AMP, adenosine 5'-monophosphate.*

tion at 4 C. The pellet was suspended and washed twice in 0.03 M sodium phosphate buffer (*p*H 7.4) at 4 C. The cells were disrupted with a Mullard ultrasonic disintegrator (60 w; 20 kc/sec) for 6 min. The debris was removed by centrifugation at 20,000 × *g* for 10 min at 4 C. The amount of protein in the cell-free extract was determined by using the Folin phenol reagent (5).

The cell-free extracts were passed through a column of Sephadex G-25 (30 by 1.5 cm) at 4 C by using sodium phosphate buffer (0.03 M, *p*H 7.4) for elution, to facilitate the removal of adenine nucleotides.

IMP dehydrogenase activity was assayed by the method described by Magasanik et al. (6) and XMP aminase activity by the method of Moyed and Magasanik (8) as modified by Udaka and Moyed (15).

Specific activities are expressed as micromoles of product formed per minute per milligram of protein.

RESULTS

Derepression in *pur* mutants blocked in AMP and GMP synthesis. A *purH* mutant has its biochemical block in purine biosynthesis prior to the formation of the intermediate IMP. This block prevents the de novo synthesis of adenine nucleotides as well as that of guanine nucleotides. Thus, when *purH* cells are grown in medium supplemented with one of the purines, they must synthesize adenine nucleotides as well as guanine nucleotides from the available purine source (cf. Fig. 1).

When fresh medium, supplemented with 2 μg of guanine per ml, was inoculated with 0.1 volume of an exponential phase *purH* culture, the syntheses of IMP dehydrogenase and XMP aminase hardly accelerate during derepression. When such a medium was inoculated with 0.001 volume of log phase cells, derepression of IMP dehydrogenase and XMP aminase occurred. (A slower exhaustion here of intra- and extracellular purines apparently favors derepression.) The pattern of derepression in a *purH* mutant, cultured under the latter condition of derepression, is presented in Fig. 2. (In all of the later experiments, 0.1 volume was always inoculated in the medium.) In addition, a derepression curve of IMP dehydrogenase in a *guaA* mutant is given in Fig. 2 for comparison.

The main differences between derepression in *purH* and derepression obtained with *gua* mutants cultured under comparable conditions are that (i) the maximal derepression in the *purH* mutant is, varying from experiment to experiment, 2 to 10 times lower than the maximum derepression in *gua* mutants and (ii) after maximal derepression in *purH*, the specific activities of IMP dehydrogenase and XMP aminase decrease rapidly to a very low level, whereas enzyme activities in *gua* mutants remain constant.

The differences in derepression between *purH* mutants and *gua* mutants are not specific for *purH*. Mutants such as *purB*, *purC*, and *purF* and the double mutants *purCguaA*, *purCguaB*, and *purAguaB* also exhibit behavior similar to *purH*. To cultures of *purB* and *purA*, mutants having an absolute requirement for adenine, also a very limited amount of adenine (1 μg/ml) was given. In comparison with *guaA* and *guaB* mutants, all these mutants have in common the additional biochemical block in the synthesis of adenine nucleotides.

The difference between these mutants and *guaA* and *guaB* mutants is that these mutants

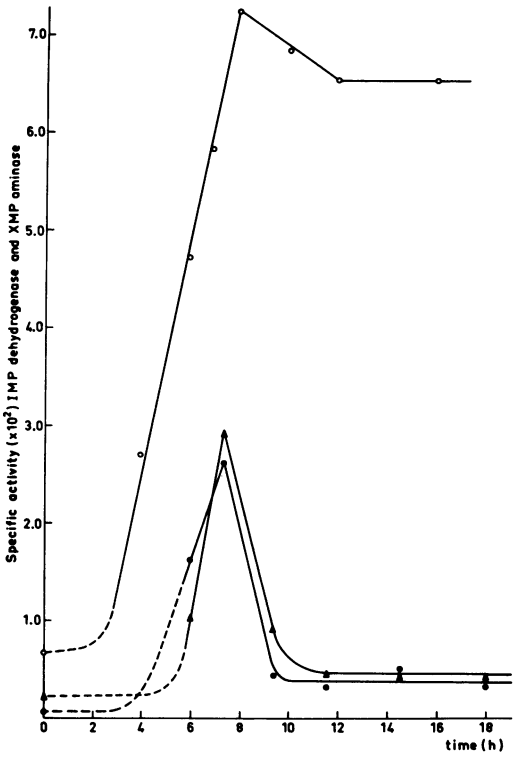

FIG. 2. *Specific activities of IMP dehydrogenase* (●) *and XMP aminase* (▲) *in a derepressed* purH *strain. Specific activity of IMP dehydrogenase in a derepressed guaA strain* (○).

cannot synthesize both adenine nucleotides and guanine nucleotides, whereas *guaA* and *guaB* mutants are blocked only in the synthesis of guanine nucleotides. The absence of adenine nucleotides in mutants blocked in adenosine 5′-monophosphate (AMP) and GMP synthesis and cultured under conditions of derepression must therefore be responsible, directly or indirectly, for the reduced maximal derepression of IMP dehydrogenase and XMP aminase and the decrease in specific activity of these enzymes after derepression. The experiments described below were performed to test the validity of this hypothesis.

Influence of adenine nucleotides on the syntheses of IMP dehydrogenase and XMP aminase. In all *pur* mutants blocked before IMP and cultured in media with 2 μg of guanine per ml, the adenine requirement cannot be abolished simply by addition of adenine without influencing the regulation of the synthesis of IMP dehydrogenase and of XMP aminase. GMP, synthesized from adenine via IMP, represses the syntheses of both IMP dehydrogenase and XMP aminase.

The effect of adenine on the syntheses of IMP dehydrogenase and XMP aminase was therefore studied in double mutants *purCguaA* and *purCguaB* to prevent the synthesis of GMP from adenine. In these mutants adenine is converted into adenine nucleotides but, because of the *gua* mutation, not into guanine nucleotides.

The effect of various concentrations of adenine on the specific activities of IMP dehydrogenase and XMP aminase in 16-hr cultures of *purCguaA* and *purCguaB* is presented in Table 2. This table shows that the specific activities of IMP dehydrogenase as well as that of XMP aminase increase when the concentrations of adenine are increased. From Table 2, it is not clear whether the increase in specific activity is due to an effect on the syntheses or on the activities of IMP dehydrogenase and XMP aminase.

The effect of adenine on the derepression of IMP dehydrogenase and XMP aminase was studied in *purCguaA* and *purCguaB* mutants, respectively, in the presence as well as in the absence of chloramphenicol. These double mutants were cultured in media supplemented with 2 μg of guanine and 12.5 μg of adenine per ml. The derepression curves in Fig. 3 illustrate the inducing effect of adenine on the activity of IMP dehydrogenase. Since chloramphenicol stops the increase of specific activity of IMP dehydrogenase, it is concluded that adenine activates in one way or another the synthesis of this enzyme. Addition of adenine to a *purCguaB* strain shows the same effect for XMP aminase, so the enhanced synthesis of XMP aminase might be coordinate with that of IMP dehydrogenase.

TABLE 2. *Effect of adenine addition on the level of IMP dehydrogenase and XMP aminase in 16-hr cultures of purCgua strains cultured under conditions of derepression (2 μg of guanine per ml of medium)*[a]

Strain	Adenine concentration in the medium (μg/ml)	Specific activity[b] × 10^3	
		XMP aminase	IMP dehydrogenase
purCguaB	0	3.5	
purCguaB	2	7.3	
purCguaB	5	17.2	
purCguaB	10	23.9	
purCguaA	0		5.4
purCguaA	2		3.4
purCguaA	5		26.3
purCguaA	10		33.7

[a] Experimental procedures have been described in the text.

[b] Expressed as micromoles of product formed per minute per milligram of protein.

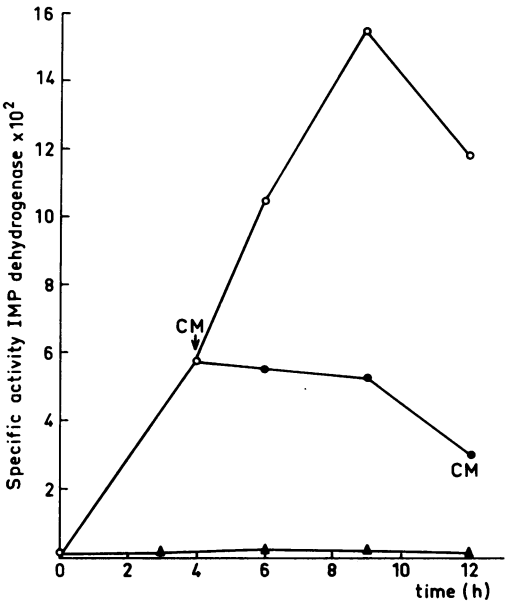

FIG. 3. *Effect of adenine (12.5 μg per ml of medium) on the synthesis of IMP dehydrogenase in a derepressed purCguaA strain. ●, adenine supplemented; ▲, adenine omitted. Chloramphenicol (CM) was added as indicated by the arrow in a final concentration of 100 μg per ml medium.*

What are the intracellular products which induce the synthesis of IMP dehydrogenase and XMP aminase? Obviously certain nucleotides are suspect of such an inducing role, e.g., adenine nucleotides or IMP and XMP which can be synthesized from extracellular adenine.

A possible role of XMP as inducer was studied in a *guaA* strain cultured under conditions of derepression (i.e. suboptimal guanine) to which xanthine was added. From xanthine, XMP is synthesized but XMP is not converted to GMP because of the *guaA* mutation. In comparison with the xanthineless control, a concentration of 4 μg of xanthine per ml of medium increased the specific activity by a factor of 2 to 7, whereas continued increases in xanthine concentration had no effect (Table 3). XMP, however, inhibits IMP dehydrogenase activity as is shown later in this paper, and, therefore, the inducing effect of xanthine addition may be the accumulation of IMP or the presence of an enlarged pool of adenine nucleotides synthesized from IMP. To obviate possible effects of the pool of IMP and adenine nucleotides, the experiment was repeated with a *purCguaA* mutant. The inducing effect of xanthine was not observed in this mutant (Table 3). Hence, it is unlikely that XMP functions as an inducer.

To exclude the possible inducing influence of IMP, a *purAguaB* mutant was isolated. This mutant was cultured in media containing suboptimal adenine and guanine concentrations and in the presence as well as the absence of hypoxanthine. IMP is synthesized from hypoxanthine, but IMP is not converted into succinyl adenosine 5-monophosphate or XMP because of the presence of the *purA* and *guaB* blocks, respectively. The synthesis of XMP aminase was not affected by the addition of hypoxanthine (Table 3).

It is concluded from the results of these experiments that one or all of the adenine nucleotides or their derivatives, but neither IMP nor XMP, act as inducer(s) for the synthesis of IMP dehydrogenase and of XMP aminase.

Since adenine nucleotides induce both IMP dehydrogenase and XMP aminase, it is possible that regulation of the expression of the *gua* operon is involved.

Influence of adenine nucleotides on the activity of IMP dehydrogenase. The experiments described below show that the lowered maximal derepression or total absence of derepression in *pur* mutants blocked in AMP and GMP synthesis is due to the lack of adenine nucleotides. Abolishing this deficiency by addition of adenine to the culture medium makes the pattern of derepression similar to that of the *gua* mutants blocked between IMP and GMP. However, in the *purH* mutant the sharp decrease in the specific activity of IMP dehydrogenase and XMP aminase after maximal derepression is not explained by these results.

Dilution of the IMP dehydrogenase and XMP aminase molecules due to growth, a possible cause of the decrease in specific activity, is not likely. The generation time of the *purH* mutant is about 2 hr. The decrease in specific activity after maximal derepression ranges from 70 to 95% for a period lasting 3 hr, a period during which the cells are no longer growing logarithmically.

IMP dehydrogenase and XMP aminase molecules may be broken down or may be nonfunctional, e.g., because of inactivation. Inactivation by 5-amino-4-imidazolecarboxamide ribonucleotide, the accumulation product of *purH* mutants is also unlikely, since *pur* mutants with other biochemical blocks before IMP show similar effects with respect to the specific activity of IMP dehydrogenase and XMP aminase. The lack of adenine nucleotides may also, directly or indirectly, cause the decrease in specific activity.

When a *purC* mutant, cultured under conditions of derepression, is supplied with adenine, a decrease in specific activity is not observed (*see* Fig. 3). From these results, one cannot conclude whether adenine nucleotides activate or prevent the breakdown of the enzyme molecules, either directly or indirectly.

TABLE 3. *Effect of purine addition to the medium on the level of IMP dehydrogenase and XMP aminase in different pur strains*

Strain	Purine added	Concn (μg/ml)	Specific activity[a] (× 10[3])	
			IMP dehydrogenase	XMP aminase
guaA	Guanine	2.5	53.9	
guaA	Guanine Xanthine	2.5 4.0	115.9	
guaA	Guanine Xanthine	2.5 20.0	115.3	
guaA	Guanine	5.0	20.2	
guaA	Guanine Xanthine	5.0 4.0	121.3	
purCguaA	Guanine	2.0	3.1	
purCguaA	Guanine Xanthine	2.0 10.0	3.4	
purAguaB	Guanine Adenine	2.0 1.0		11.9
purAguaB	Guanine Adenine Hypoxanthine	2.0 1.0 5.0		9.8
purAguaB	Guanine Adenine Hypoxanthine	2.0 1.0 10.0		12.2

[a] Expressed as micromoles of product formed per minute per milligram of protein.

To distinguish between a breakdown or an inactivation mechanism, the following experiment was performed. A *purH* mutant was cultured under conditions of derepression (2 μg of guanine per ml). At 12 hr later, when the decrease in specific activity had already taken place, the medium was supplemented with an excess of adenine (final concentration 25 μg per ml), and the specific activity was studied for several hours. If the enzyme molecules are broken down, there should be no increase in activity, since an excess of adenine in the culture medium of the *purH* mutant represses the syntheses of IMP dehydrogenase and XMP aminase. If, on the other hand, there is inactivation of the enzyme molecules, one can expect an increase in enzyme activity, assuming a reversible process is involved. Fig. 4 shows an increase in the activity of XMP

aminase after adenine addition. The addition of chloramphenicol did not prevent this increase. IMP dehydrogenase is a less suitable enzyme to study in this experiment, since the GMP pool would be enlarged by the excess of adenine in the medium. Furthermore it is known that GMP inhibits the activity of IMP dehydrogenase (7).

The results of this experiment suggest that the absence of adenine nucleotides is responsible for the decrease in specific activity in the derepressed *purH* mutant, probably by inactivation. Addition of adenine may prevent inactivation directly or indirectly. Addition of guanine also has the same effect, owing to the conversion of guanine into adenine nucleotides (H. J. J., Nijkamp, Ph.D. Thesis, State University, Utrecht, The Netherlands, 1968). Inactivation may be prevented indirectly by promoting the synthesis of an activator of an inhibitor, or directly by activating IMP dehydrogenase and XMP aminase or by competing with an inhibitor of these two enzymes.

A possible direct influence of adenine nucleotides on the activity of IMP dehydrogenase was studied in vitro. The influence of AMP was studied in cell-free extracts of *purH* cells cultured under conditions of derepression. These cells were harvested at a time corresponding to low level of enzyme activity (i.e. 10 to 12 hr). Table 4 shows the effect of several concentrations of

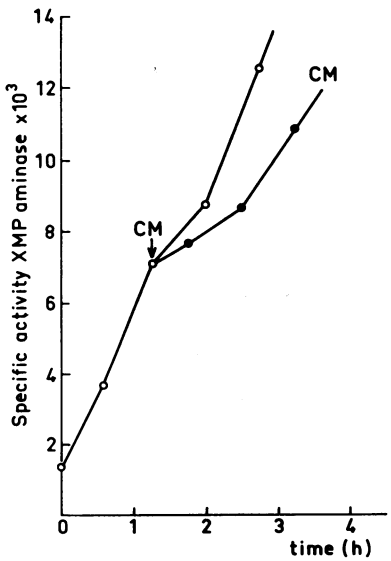

FIG. 4. *Effect of adenine (25 μg per ml of medium) on the activity of XMP aminase. The adenine was added to a 12 hr (zero time) culture of a derepressed purH strain. CM was added as indicated by the arrow in a final concentration of 100 μg per ml of medium.*

TABLE 4. *Effect of AMP on the activity of IMP dehydrogenase in a cell-free extract of a derepressed purH strain*[a]

AMP (μmoles)	Specific activity[b] of IMP dehydrogenase (× 10³)	Relative activity
		%
0	1.2	100
0.25	1.8	150
0.50	2.7	225
1.0	1.5	125
2.0	1.4	117
20.0	0.0	0

[a] The reaction mixtures contained 0.1 μmole of IMP. The remaining composition of the mixtures (final volume 3 ml) was unchanged.

[b] Expressed as micromoles of product formed per minute per milligram of protein.

AMP on the activity of IMP dehydrogenase. From the values indicated, it became evident that AMP (<2 μmoles) activates IMP dehydrogenase. This activating effect becomes an inhibitory effect at higher concentrations. The maximal activation in this experiment corresponds to a factor of 2.2, at the concentration of 0.5 μmole of AMP. Maximal activation varied from experiment to experiment with the AMP concentrations used. The activation factor of 3.5 was the highest activation ever measured. The specific activities of IMP dehydrogenase in the experiment described above were too low to be measured accurately. Therefore, further experiments were conducted with a *guaA* mutant. These *guaA* mutants are blocked in the synthesis of GMP exclusively. Hence, a lack of adenine nucleotides will not appear in these cells while cultured under conditions of derepression (2 to 5 μg of guanine per ml of medium). Molecules of IMP dehydrogenase, in this case, exist in an activated state. Figure 5 shows the effect of 0.5 μmole and 15 μmoles of AMP on the activity of IMP dehydrogenase as a function of the IMP concentration, i.e., activation fails to occur. An inhibitory effect appears at a concentration of 0.5 μmole of AMP. ADP and adenosine triphosphate (ATP) exhibit similar effects. Adenine nucleotides inhibit the activity of IMP dehydrogenase, especially at higher concentrations.

When adenine nucleotides are withdrawn from the cell-free extracts of derepressed *guaA* cultures, it is possible to simulate, in vitro, the inactivation as it appeared in vivo in a derepressed *purH* mutant. For this purpose, the cell-free extracts had to be submitted to gel filtration or dialysis. Either method results in an inactivation of IMP dehydrogenase of about 50 to

70%. Fig. 6 shows the effect of AMP added to a gel filtration-treated, cell-free extract of a derepressed *guaA* mutant on IMP dehydrogenase activity as a function of the IMP concentration. The reactivation observed at 0.5 μmole of AMP and the inhibition observed at 15 μmoles of AMP are in agreement with data obtained by using the cell-free extract of a *purH* mutant (*see* Table 4). No explanation could be given for the irregularity in the curves corresponding to the IMP concentration of 0.05 μmole (Fig. 6).

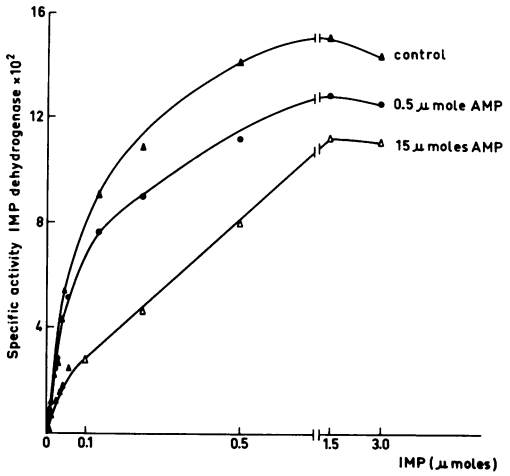

FIG. 5. *Effect of AMP on the activity of IMP dehydrogenase in a cell-free extract of a derepressed guaA strain as a function of the IMP concentration. The final volume of the reaction mixture was 3 ml.*

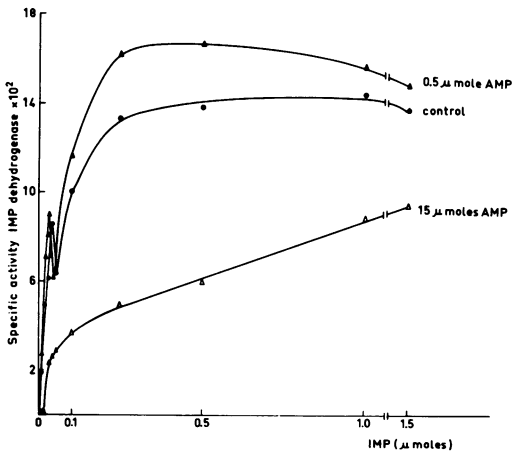

FIG. 6. *Effect of AMP on the activity of IMP dehydrogenase in a gel-filtration-treated, cell-free extract of a derepressed guaA strain. The specific activity of IMP dehydrogenase prior to gel filtration was 0.044. The inactivation of IMP dehydrogenase, effected by passing through Sephadex G-25, was 62%. The final volume of the reaction mixtures was 3 ml.*

Table 5 lists the effects of several purine derivatives on enzyme activity. AMP, ADP, ATP, and adenosine each reactivates IMP dehydrogenase. The reactivation by AMP here is more pronounced than that presented in Fig. 6, although the same cell-free extract was used. The reason is that inactive IMP dehydrogenase molecules are, unlike active molecules, very unstable; the experimental data in Fig. 6 were obtained later than those in Table 5. Inosine, XMP (product inhibition), GTP, and GMP (end product inhibition) each inhibits the activity of IMP dehydrogenase. Similar results were obtained in dialysis experiments (H. J. J. Nijkamp, Ph.D. Thesis, State University, Utrecht, The Netherlands, 1968).

DISCUSSION

Derepression of the synthesis of IMP dehydrogenase and of XMP aminase in derepressed *pur* mutants biochemically blocked in AMP and GMP synthesis differs from derepression in mutants blocked only in GMP synthesis. In *pur* mutants blocked in the synthesis of AMP and GMP, the derepression is less pronounced; maximal derepression is lower than that of *gua* mutants. In addition, after maximal derepression is reached, there is a sharp decrease in the specific activity of IMP dehydrogenase and of XMP aminase. In *gua* mutants this phenomenon does not appear.

TABLE 5. *Effect of purine derivatives on the activity of IMP dehydrogenase in a gel-filtered cell-free extract of a derepressed guaA strain*[a]

Purine derivatives	Concentration (μmoles)	Specific activity[b] of IMP dehydrogenase (× 10³)	Relative activity
			%
None	0	27.0	100
AMP	0.5	42.3	157
ADP	0.5	39.2	145
ATP	0.5	45.3	168
Adenosine	0.5	36.0	133
Inosine	0.5	21.6	80
XMP	0.1	10.8	40
GMP	0.2	12.1	45
GTP	0.2	24.6	91

[a] The reaction mixture (final volume 3 ml) contained 0.1 μmole of IMP. The remaining composition was unchanged. The specific activity of IMP dehydrogenase prior to gel filtration was 0.044. The inactivation of IMP dehydrogenase, effected by passing through Sephadex G-25, was 62%.

[b] Expressed as micromoles of product formed per minute per milligram of protein.

The results in this paper indicate that the lack of adenine nucleotides is responsible for the two abnormal derepression phenomenona in *pur* mutants blocked in AMP and GMP synthesis. It has been demonstrated that adenine nucleotides play a dual role in the regulation of GMP synthesis; they affect the syntheses as well as the activities of IMP dehydrogenase and XMP aminase.

Adenine nucleotides induce the synthesis of IMP dehydrogenase and of XMP aminase. The mechanism of induction may therefore involve the expression of the *gua* operon. Furthermore, it is unlikely that IMP and XMP, both nucleotides having been synthesized from adenine, induce the synthesis of these enzymes (Table 3).

It is already known that the syntheses of both IMP dehydrogenase and XMP aminase are susceptible to the repressive action of guanine nucleotides (10). Therefore the synthesis of the products of the *gua* operon is controlled by two systems: (i) repression by guanine nucleotides and (ii) induction by adenine nucleotides.

It is necessary to discuss the specificity of the induction by adenine nucleotides. Starvation of purine nucleotides in *pur* mutants blocked in AMP and GMP synthesis will restrict RNA and protein synthesis. Besides this general effect there should be an additional specific effect on the expression of the *gua* operon. If purine starvation limits the synthesis of all RNA molecules at the same rate in these mutants, one would still expect a derepression of IMP dehydrogenase and XMP aminase synthesis. This is not the case. When a *purH* culture is diluted 1 to 10 in medium supplemented with 1 to 4 μg of guanine, there is no derepression at all, although a limited amount of both adenine and guanine nucleotides can be synthesized from the available guanine. This result together with the results of Hoekstra and Nijkamp (*unpublished data*) presented below indicate that a special adenine nucleotide, not synthesized under the starvation conditions used, might be involved in the expression of the *gua* operon.

There are indications that the inducing effect of adenine nucleotides is not restricted to the *gua* operon only. Alkaline phosphatase synthesis also needs an adenine nucleotide inducer (Wilkins and Gallant, *manuscript in preparation*). More recently, it was observed (Hoekstra and Nijkamp, *unpublished results*) that in vivo cyclic 3',5'-AMP increased the levels of both IMP dehydrogenase and XMP aminase very markedly. Cyclic 3',5'-AMP induced about 20 times better than 5'-AMP did under similar conditions. In *E. coli* probably the syntheses of the anabolic enzymes IMP dehydrogenase and XMP aminase mimic those of the catabolic enzymes such as β-galactosidase (12, 16) and tryptophanase (12) in their response to the regulatory action of cyclic 3',5'-AMP. It was observed earlier by Kuramitsu et al. (4) that, in the *pur* double mutant they used (B-22-G), an increase in the intracellular pool of adenine nucleotides results in a stimulation of nucleic acid synthesis. This stimulation caused a twofold induction of IMP dehydrogenase because of further depletion of the pool of guanine derivatives under their conditions. Although not pertinent in all the experiments described in this paper, this effect may play a role here too, together with the possible inducing effects discussed above.

Not only do adenine nucleotides affect the synthesis of IMP dehydrogenase and XMP aminase, but they also exhibit a profound effect on the activities of these enzymes.

Results of in vivo experiments indicate that, after derepression, the decrease in specific activity of IMP dehydrogenase and XMP aminase in *pur* mutants blocked in AMP and GMP synthesis was due to inactivation of these enzymes. In vitro experiments showing the inactivation of IMP dehydrogenase by the exclusion of adenine nucleotides and those showing the reactivation of the inactivated enzyme molecules after the addition of these nucleotides are in full agreement with the in vivo results. The observed inhibition of IMP dehydrogenase by adenine nucleotides at higher (>2 μmoles) concentrations is probably due to the affinity of adenine nucleotides for the substrate (IMP) site or feedback (GMP) site of this enzyme or might be an expression of competition at the nicotinamide adenine dinucleotide site. Adenine nucleotides are known to inhibit dehydrogenases mediated by pyridine nucleotides (9).

IMP dehydrogenase activity is inhibited by XMP as is shown in Table 5. Furthermore, it is assumed from the results of the in vivo experiments that adenine nucleotides in physiological concentrations are also metabolic activators of XMP aminase. It has been shown that the antibiotic psicofuranine, a derivative of adenosine, which noncompetitively inhibits XMP aminase activity, binds to a specific site or region of XMP aminase (2), thereby modifying its protein structure (3). The metabolic function of this site may be a regulatory one (2). Since Fukuyoma (*unpublished results, see* reference 3) has shown that AMP binds to XMP aminase, it can be said from our results that AMP binds to this site, thereby activating the XMP aminase molecules.

The regulatory functions of adenine nucleotides in the biosynthesis of GMP, namely, the induction and the activation of IMP dehydrogenase and XMP aminase, are of physiological importance. They maintain a balance between the synthesis of adenine nucleotides and guanine nucleotides. An acceleration of the synthesis of adenine nucleotides results in an enhanced GMP synthesis by the induction and activation of IMP dehydrogenase and XMP aminase. A deficiency in adenine nucleotides causes exactly the reverse of these phenomena.

In summary, the regulatory principles in the biosynthesis of GMP from IMP are the following: (i) repression of the synthesis of IMP dehydrogenase and XMP aminase by guanine nucleotides, (ii) induction and (iii) activation of these enzymes by adenine nucleotides, (iv) feedback inhibition of IMP dehydrogenase by GMP (7), and (v) product inhibition of this enzyme by XMP.

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