Biochimica et Biophysica Acta, 527 (1978) 485—496

BBA 68617

EFFECTS OF AMINO ACIDS, ADENINE NUCLEOTIDES AND INORGANIC PYROPHOSPHATE ON GLUTAMINE SYNTHETASE FROM *ANABAENA CYLINDRICA*

S.K. SAWHNEY and D.J.D. NICHOLAS

Department of Agricultural Biochemistry, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia (Australia)

(Received May 9th, 1978)

Summary

Glutamine synthetase (L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2) from *Anabaena cylindrica* was inhibited by alanine, glycine, serine and aspartate. The effects of alanine and serine were uncompetitive with respect to glutamate, while those of glycine and aspartate were non-competitive and mixed type respectively. Different pairs of amino acids and their various combinations caused a cumulative inhibition of the enzyme activity. Glutamine synthetase was also inhibited by ADP and AMP and both nucleotides affected the enzyme competitively with respect to ATP and non-competitively for glutamate. Inorganic pyrophosphate, between 2 and 3 mM, produced a very pronounced inhibition of enzyme activity. The inhibition by PP_i was uncompetitive for ATP. Various combinations of the adenine nucleotides, PP_i and P_i exerted a cumulative inhibitory effect on the enzyme activity, as did the amino acids, in different combinations with either adenine nucleotides, PP_i or P_i.

The effects of the adenine nucleotides and the amino acids were more pronounced at higher concentrations of ammonia. Except for serine similar responses of these effectors were obtained with increasing concentrations of Mg^{2+}. It is proposed that changes in the free concentrations of Mg^{2+} are important in energy-dependent regulation of the enzyme activity in this alga.

Introduction

It is well established that under conditions of a limited supply of ammonia, inorganic nitrogen is assimilated via glutamine synthetase/glutamate synthase pathway [1—8]. Glutamine synthetase (L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2) is thus the key enzyme in assimilation of ammonia and it also represents the first step in the production, through highly branched metabolic sequences, of a variety of cellular nitrogenous constituents [9,10].

The requirements by various pathways for glutamine necessitate regulation of glutamine synthetase activity so that synthesis of this metabolite is controlled in accordance with these demands. Synthesis of this enzyme in microorganisms has been shown to be influenced by the source of nitrogen in the growth media [11–13]. In addition, in *Escherichia coli* and some other gram-negative bacteria, a more rapid control of glutamine synthetase is effected through reversible adenylation of the enzyme [14]. However, in other organisms [12,15,16] attempts to demonstrate such an adenylation have not been successful. In these organisms, therefore, regulation of the enzyme activity by feedback inhibition is presumably more important. Glutamine synthetase from different sources is inhibited by a variety of metabolites including AMP, CTP, histidine, tryptophan, glycine, alanine, glucosamine 6-phosphate and carbamyl phosphate. The enzyme from different organisms, however, varies in its sensitivity to the metabolites synthesized via the glutamine-dependent pathways [17,18]. In contrast to the extensive investigations on the enzyme from bacterial and animal sources, only limited information is available [12,19,20] about the regulatory properties of this enzyme from blue-green algae. We have, therefore, examined the effects of various amino acids, organic acids, nucleotide and pyrophosphate on the activity of glutamine synthetase from *Anabaena cylindrica*.

Materials and Methods

Materials

A. cylindrica (Lemmerman, 1403/2A) was obtained from the Culture Collection of Algae and Protozoa, The Botany School, Cambridge, U.K. The various nucleotides, L-serine, L-glutamic acid, L-glutamine and γ-glutamylhydroxamic acid were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. L-alanine and L-aspartic acid were bought from Mann Research Laboratories Inc., New York, U.S.A. and L-glycine was from B.D.H., Poole, U.K. Tetrasodium pyrophosphate was from Ajax Chemicals Ltd., Sydney, Australia, L-[U-^{14}C]glutamic acid was from The Radiochemical Centre, Amersham, Bucks., U.K.

Methods

Preparation of cell-free extracts. 7-day-old cultures of *A. cylindrica*, grown in a medium containing 5 mM KNO_3, were harvested as described earlier [21]. After washing three times with 0.1 M Tris-HCl (pH 7.5), 10 mM $MgCl_2$, the cells were broken by passing them twice through a chilled French pressure cell at 20 000 lbs/in^2. The homogenate was centrifuged at 6000 × *g* for 20 min and the supernatant thus obtained was used for the purification of glutamine synthetase.

Enzyme assays. The effects of various metabolites were examined on both the γ-glutamyltransferase and the biosynthetic activities of glutamine synthetase.

The Mn^{2+}-dependent γ-glutamyltransferase was determined from the rate of γ-glutamylhydroxamate produced as described by Shapiro and Stadtman [22]. The reaction mixture in a final volume of 1 ml contained: 40 μmol imidazole-HCl buffer (pH 7.0), 30 μmol L-glutamine, 30 μmol hydroxylamine, 3 μmol $MnCl_2$, 2 μmol sodium arsenate, 0.4 μmol ADP and 0.05 ml (containing 4 μg

protein) purified enzyme. The amount of γ-glutamylhydroxamate produced after 20 min at 37°C was determined spectrophotometrically [22]. Under these conditions, the formation of γ-glutamylhydroxamate was linear over a 40-min incubation period.

Except for the experiments in which the effect of P_i was examined, the Mg^{2+}-dependent biosynthetic activity of glutamine synthetase was determined from the rate of release of P_i [22]. The reaction mixture in a final volume of 0.3 ml contained: 10 μmol imidazole-HCl buffer (pH 7.0), 2.4 μmol L-glutamate, 20 μmol NH_4Cl, 20 μmol $MgCl_2$, 1.5 μmol ATP and 0.05 ml (containing 4 μg protein) purified enzyme. Either glutamate or NH_4Cl was omitted from the control tubes. Any modifications in the composition of this reaction mixture are specified in the individual experiments. After 30-min incubation at 37°C, the amount of P_i formed was determined [22]. The rate of P_i production was linear up to 45 min.

When studying the effect of P_i on the biosynthetic activity, the rate of synthesis of [^{14}C]glutamine from [^{14}C]glutamate was followed. The reaction mixture in a final volume of 0.3 ml contained 10 μmol imidazole-HCl buffer (pH 7.0), 20 μmol $MgCl_2$, 10 μmol NH_4Cl, 1.5 μmol ATP, 1.2 μmol [U-^{14}C]-glutamate (0.15 μCi) and 0.05 ml (4 μg protein) purified enzyme. Tubes without NH_4Cl served as controls. After 30-min incubation at 37°C, the reaction was stopped by adding 0.2 ml ice-cold 90% (v/v) ethanol. After centrifuging at 3000 × *g* for 20 min, 0.1 ml of the supernatant fraction was spotted onto 3 MM Whatman paper. The [^{14}C]glutamate and [^{14}C]glutamine were separated by high-voltage electrophoresis, in 0.1 M citrate buffer (pH 5.0) at 1500 V for 1 h in an apparatus designed by Tate [24]. After drying, these compounds were located on the electrophoretograms from the ninhydrin spots of the standards of glutamate and glutamine run along with the samples. The areas corresponding to glutamate and glutamine were cut into 2.5 cm long strips, placed in scintillation vials containing 2 ml scintillation fluor (0.03% (w/v) POPOP and 0.3% (w/v) PPO in toluene) and counted in a Packard Tri Carb Liquid Scintillation spectrometer (Model 3375). The counting efficiency for ^{14}C was 64%. The biosynthetic activities determined by either the release of P_i or the synthesis of [^{14}C]glutamine were identical.

Other enzyme assays. Adenylate kinase and 5′-nucleotidase were assayed as described previously [21]. Inorganic pyrophosphatase and glutaminase were determined by the procedures of Schwenn et al. [25] and Wood et al. [26], respectively.

Protein measurements. The amount of protein in the enzyme preparation was determined by the procedure of Lowry et al. [27] using bovine serum albumin as standard.

Enzyme purification. Glutamine synthetase from the cell-free extracts of *A. cylindrica* was purified about 30-fold by the method described earlier [23]. The purification procedure involved heat treatment at 50°C for 30 min, pH fractionation, $(NH_4)_2SO_4$ precipitation and gel chromatography through a Sepharose 6B column. Specific activity of the purified glutamine synthetase was 240 μmol γ-glutamylhydroxamate produced/mg protein in 15 min. The purified enzyme when stored at 4°C was stable for several months.

Results

A 30-fold-purified glutamine synthetase from *A. cylindrica*, prepared as described elsewhere [23], was used throughout these investigations. This preparation was free of 5′-nucleotidase, adenylate kinase, inorganic pyrophosphatase and glutaminase activities.

Effects of amino acids

The effects of various L-amino acids on both the Mg^{2+}-dependent biosynthetic and Mn^{2+}-dependent γ-glutamyltransferase activities of glutamine synthetase were examined. The biosynthetic activity was significantly inhibited by alanine, glycine, serine and aspartate. At 5 mM, these amino acids diminished the enzyme activity by 49, 42, 33 and 19 per cent respectively. Citrulline and glutamine, at 10 mM, lowered the enzyme activity by 22 and 10% respectively. The γ-glutamyltransferase activity was inhibited by 5 mM of alanine (88%), glycine (50%), serine (30%), aspartate (20%), asparagine (20%), glutamate (23%) and citrulline (15%). The following amino acids, up to 10 mM, did not affect the biosynthetic or the γ-glutamyltransferase activities: tryptophan, histidine, lysine, cysteine, cystine, leucine, isoleucine, methionine, phenylalanine, threonine, tyrosine, valine and arginine.

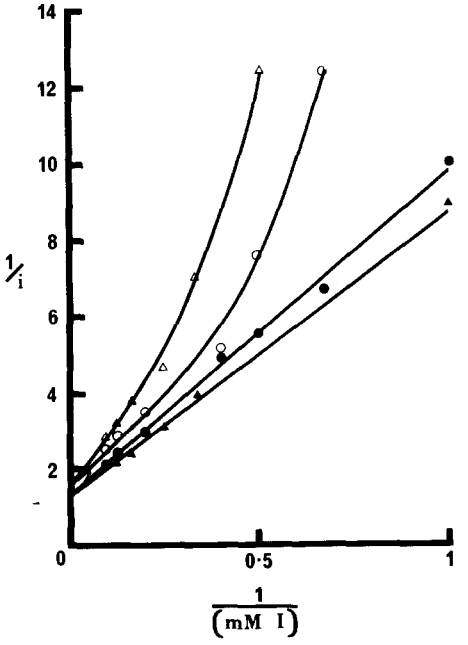

Fig. 1. Effect of various concentrations of alanine, aspartate, glycine and serine on enzyme activity. Double-reciprocal plot of enzyme inhibition at various concentrations of the four amino acids (0—10 mM). Biosynthetic activity was determined from the rate of P_i production. The reaction mixture in a final volume of 0.3 ml contained: 10 μmol imidazole HCl (pH 7.0), 2.4 μmol L-glutamate, 10 μmol NH_4Cl, 20 μmol $MgCl_2$, 3 μmol ATP, 0.05 ml purified enzyme. Percent inhibition was calculated on the basis of the activity of the control sample which was 0.59 μmol P_i produced in 30 min. ▲, L-alanine; ●, L-aspartate; ○, L-glycine; △, L-serine.

The effects of different concentrations of alanine, aspartate, glycine and serine on the biosynthetic activity was examined. At equimolar concentrations alanine and glycine suppressed enzyme activity to almost the same extent and were more effective than either aspartate or serine. To ascertain whether these amino acids at their saturating concentrations resulted in a complete inhibition of enzyme activity, the data plotted as double reciprocal plots of the fractional inhibition against the concentration of the inhibitor [28]. In this plot a complete inhibition at the saturating concentrations of the modifier is indicated when the curve intersects the y axis at a value of <1. Since the intercepts for all the four amino acids were >1 (Fig. 1), these effectors only partially inhibited the enzyme activity.

The kinetics of inhibition of these amino acids in the presence of varying concentrations of substrates were then examined. Alanine and serine inhibited the activity uncompetitively with respect to glutamate, while glycine and aspartate showed non-competitive and mixed type inhibition respectively. The results in Fig. 2 indicate that inhibition by these amino acids was more pronounced at higher concentrations of ammonia. Thus, 5 mM of either alanine or glycine reduced enzyme activity by less than 5% at 1 mM ammonia, but the activity was suppressed by about 45% on increasing the concentration of ammonia to 5 mM. Similarly, the inhibition by either alanine, glycine or aspartate was progressively accentuated as the concentration of Mg^{2+} was increased from 5 to 50 mM (Fig. 3). Inhibition by serine, however, remained constant at all

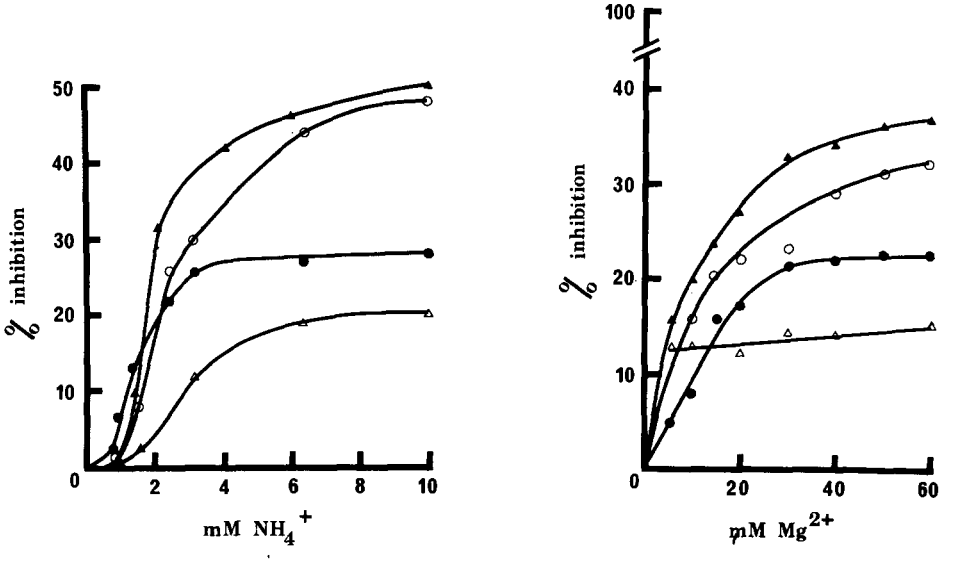

Fig. 2. Effects of amino acids at varying concentrations of ammonia. Details of the precedure and reaction mixture as described in Fig. 1 except that the concentration of NH_4Cl in the reaction mixture was varied as indicated and it contained 1.5 μmol ATP and 5 mM of the following amino acids: ▲, L-alanine; ○, L-glycine; ●, L-serine; △, L-aspartate.

Fig. 3. Inhibition of enzyme activity by amino acids at varying concentrations of Mg^{2+}. Details of the experimental procedure as in Fig. 1, except that the reaction mixture contained 5 mM of the specified amino acid, 1.5 μmol ATP, 10 μmol glutamate and the concentrations of Mg^{2+} as indicated. ▲, alanine; ○, glycine; ●, aspartate; △, serine.

TABLE I

COMBINED EFFECTS OF VARIOUS AMINO ACIDS ON GLUTAMINE SYNTHETASE

The enzyme activity was determined from the rate of P_i production. The reaction mixture in a final volume of 0.3 ml contained: 10 μmol imidazole-HCl (pH 7.0), 20 μmol $MgCl_2$, 7.5 μmol NH_4Cl, 3 μmol ATP, 1.5 μmol Glutamate, 0.05 ml purified enzyme and 5 mM of each of the indicated amino acids. In control sample 0.39 μmol P_i were produced in 30 min. Values for cumulative inhibition were calculated by the procedure of Woolfolk and Stadtman [29]. Figures in the parenthesis are per cent inhibition by individual amino acid.

Inhibitor (5 mM each)	Inhibition (%) calculated for		
	Observed	Cumulative	Additive
Gly (42) + Ser (32)	58	58	74
Gly (42) + Asp (26)	49	57	68
Gly (42) + Ala (49)	61	70	91
Ser (32) + Ala (49)	62	65	81
Ser (32) + Asp (26)	45	49	58
Ala (49) + Asp (26)	59	62	75
Gly (42) + Ser (32) + Ala (49)	64	79	123
Ala (49) + Ser (32) + Asp (26)	62	74	107
Gly (42) + Ala (49) + Asp (26)	68	77	117
Gly (42) + Ala (49) + Asp (26) + Ser (32)	74	84	149

the concentrations of Mg^{2+}. The double reciprocal plots of the enzyme activity against Mg^{2+} concentration gave uncompetitive inhibition by alanine, glycine and aspartate. The extent of inhibition of the enzyme activity by these amino acids was not altered on varying the concentration of ATP over a range of 2—10 mM.

The combined effects of various amino acids on the enzyme activity are presented in Table I. The values for cumulative inhibition were calculated by the method of Woolfolk and Stadtman [29]. Inhibitions by the different combinations of these amino acids were invariably lower than the sum of inhibitions caused by the amino acids individually. For all the possible combinations examined, the observed inhibitions were in fairly good agreement with the values calculated for their cumulative inhibitory effects.

Effect of organic acids

Pyruvate, α-ketoglutarate, succinate, fumarate, malate, oxaloacetate, oxalate, glycollate and glyoxylate, up to 10 mM, affected the biosynthetic activity by less than 5%. Pyruvate, α-ketoglutarate and oxaloacetate at 10 mM each, however, inhibited the Mn^{2+}-dependent γ-glutamyltransferase activity by 50, 64 and 67%, respectively. Their mechanism of inhibition was not, however, investigated further.

Effect of nucleotides

The effects of various nucleotides, at 10 mM each, on glutamine synthetase were also examined. Among the nucleotide monophosphates, 5′-AMP inhibited the biosynthetic activity by 40%, whereas 3′-AMP, GMP, CMP, UMP, TMP and IMP were ineffective. The Mn^{2+}-dependent γ-glutamyltransferase activity was

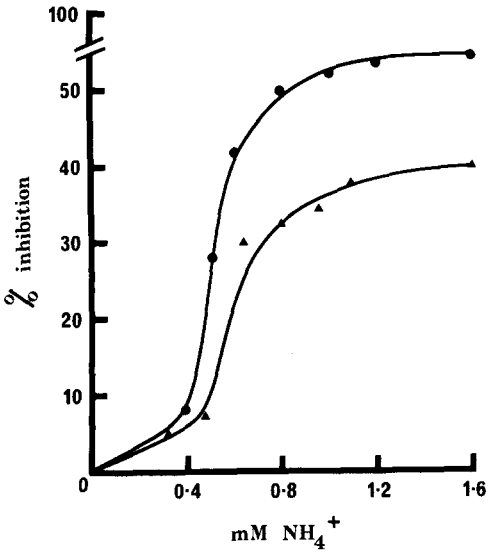

Fig. 4. Effect of varying concentrations of ammonium chloride on the inhibition by ADP and AMP. Experimental conditions as in Fig. 1 except that the concentration of NH_4Cl was varied as shown in the figure. The concentration of adenine nucleotides in the reaction mixture were: ▲, 10 mM ATP; ●, 4 mM ADP.

less sensitive to 5′-AMP than the biosynthetic activity and was diminished by only 5%. However, GMP, IMP and TMP inhibited the γ-glutamyltransferase reaction by 15, 20 and 30%, respectively; 5′-ADP lowered the biosynthetic activity by 80%, whereas GDP, CDP, UDP, IDP, GTP, TTP, CTP and ITP were without effect (less than 5%). Except for ATP, which produced a 50% inhibition, none of the other nucleotide di- or triphosphates had any effect on the γ-glutamyltransferase activity. Thus, among the various nucleotides examined, only 5′-AMP and ADP significantly inhibited the biosynthetic activity. The inhibition by either of these nucleotides increased progressively at their higher concentrations (1—10 mM). The enzyme was much more sensitive to ADP than AMP. Thus, the enzyme activity was lowered by 50% in the presence of 2 mM ADP, whereas only a 40% inhibition was obtained with 10 mM AMP. The curves for double reciprocal plots of the fractional inhibition against concentrations of either ADP or AMP intersected the y axis at 0.9, thus indicating that at saturating concentrations both adenine nucleotides completely inhibited the biosynthetic activity. Lineweaver-Burk plots of enzyme activity at varying concentrations of ATP indicated that both ADP and AMP inhibited the activity competitively with respect to ATP. These adenine nucleotides gave non-competitive inhibition with glutamate. Their effects against Mg^{2+} were uncompetitive, thus suggesting that either a prior binding of Mg^{2+} to the enzyme was essential for their action or these nucleotides act as their Mg^{2+}-nucleotide complexes. As shown earlier for the amino acids, the extent of inhibition by these nucleotides increased at higher concentrations of ammonia (Fig. 4). Thus ADP and AMP, at less than 0.4 mM ammonia, depressed enzyme activity by less than 7%, but the inhibition by ADP and AMP increased progressively to 32 and 50%, respectively as the concentration of ammonia was increased to 0.8 mM.

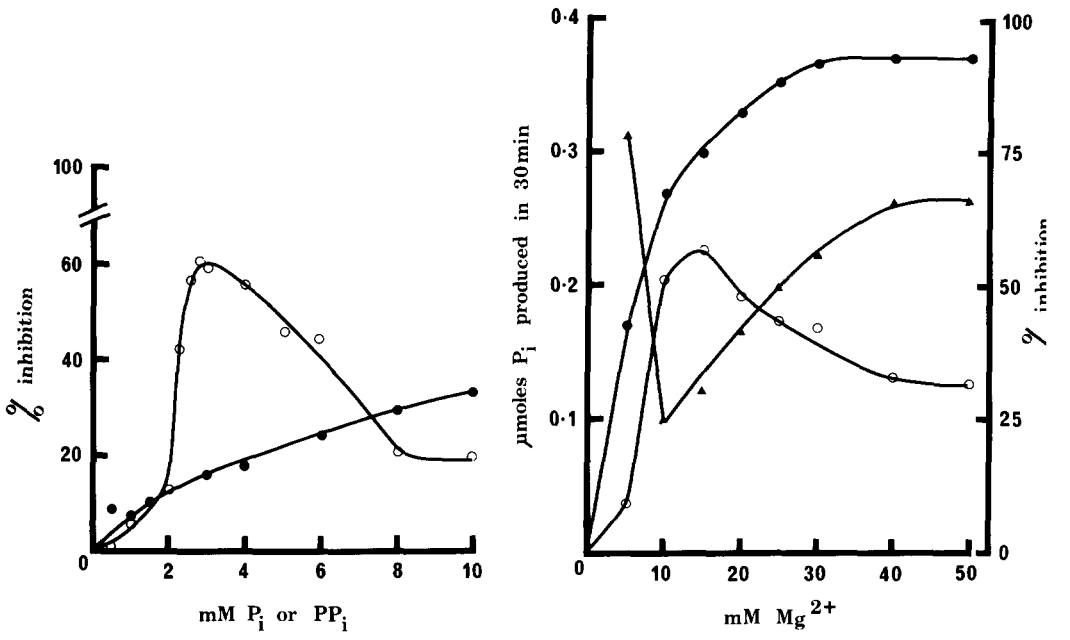

Fig. 5. Effects of different concentrations of inorganic pyrophosphate and phosphate on enzyme activity. For the experiment with inorganic pyrophosphate the details of the procedure were as described in Fig. 1, except that the reaction mixture contained 3 μmol glutamate, and the specified concentrations of neutralized tetrasodium pyrophosphate. For the experiment with phosphate, the enzyme activity was determined from the rate of synthesis of [^{14}C]glutamine from [U-^{14}C]glutamate. In the control samples 0.41 μmol P_i were produced and 0.40 μmol [^{14}C]glutamine were formed in 30 min in the experiments for PP_i and P_i respectively. ○, PP_i; ●, P_i.

Fig. 6. Inhibition of enzyme activity by inorganic pyrophosphate at varying concentrations of Mg^{2+}. Experimental procedure as in Fig. 5, except that the concentration of Mg^{2+} was varied as shown and where indicated 2.2 mM PP_i was included in the assay mixture. ●, enzyme activity of control sample; ○, enzyme activity in presence of 2.2 mM PP_i; ▲, % inhibition.

Effects of phosphate and pyrophosphate

The biosynthetic activity of glutamine synthetase was suppressed by increasing concentrations of P_i and at 10 mM, it lowered the activity by 32%. The response of the enzyme to increasing concentrations of PP_i was, however, complex (Fig. 5). Thus, at 2 mM, PP_i inhibited by about 10% but a slight further increase in its concentration markedly depressed enzyme activity. A precipitate was formed at concentrations in excess of 3 mM PP_i and at these higher concentrations its inhibitory effect steadily declined, probably due to the precipitation of Mg^{2+} pyrophosphate. The double reciprocal plot of the effect of PP_i against the increasing concentrations of ATP indicated an uncompetitive inhibition with respect to ATP. In contrast, P_i acted as a non-competitive inhibitor. The data in Fig. 6 show that glutamine synthetase was inhibited by about 80% by 2.2 mM PP_i in the presence of 5 mM Mg^{2+}. However, on increasing Mg^{2+} to 10 mM the inhibition was reduced to 25%. This marked inhibition of the enzyme by PP_i at 5 mM Mg^{2+} was probably due to a limited availability of Mg^{2+} for the enzyme activity associated with the formation of a Mg^{2+} complex with PP_i. The inhibition by PP_i, however, increased progressively as the concentra-

TABLE II

COMBINED EFFECTS OF AMINO ACIDS AND ADENINE NUCLEOTIDES ON GLUTAMINE SYNTHETASE

The experimental details as in Table I except that the assay mixture contained 2.4 μmol ATP and, wherever indicated, 5 mM specified amino acids and 1.5 mM ADP. Activity of the control sample was 0.40 μmol P_i produced in 30 min.

Inhibitor (5 mM each *)	Inhibition (%) calculated for		
	Observed	Cumulative	Additive
ADP (28) + Ser (26)	46	46	54
ADP (28) + Gly (34)	52	52	62
ADP (28) + Ala (46)	59	61	74
ADP (28) + Asp (19)	43	41	84
ADP (28) + Gly (34) + Ser (26)	57	64	88
ADP (28) + Asp (19) + Ala (46)	62	68	93
ADP (28) + Ser (26) + Ala (46)	64	70	100
ADP (28) + Gly (34) + Asp (19)	57	61	81
ADP (28) + Gly (34) + Asp (19) + Ala (46) + Ser (26)	71	83	153

* Except for ADP which was 1.5 mM.

tion of Mg^{2+} was further increased from 10 to 40 mM. The results thus indicate that the inhibition by PP_i was accentuated at higher concentrations of Mg^{2+}.

Combined effects of various metabolites

The nature of inhibition of the biosynthetic activity by various metabolites in the presence of each other was investigated. Results in Table II show that ADP when tested with the various amino acids, either singly or in combination, inhibited the enzyme cumulatively. Similar results were obtained when ADP was replaced by either AMP, PP_i or P_i. The combined effects of PP_i, P_i and

TABLE III

COMBINED EFFECTS OF ADENINE NUCLEOTIDE, PYROPHOSPHATE AND PHOSPHATE ON GLUTAMINE SYNTHETASE

The enzyme activity was determined from the rate of [^{14}C]glutamine production. The reaction mixtures contained the following compounds as indicated: 1.5 mM ADP, 5 mM AMP, 2.30 mM PP_i and 10 mM P_i. In the control sample 0.4 μmol [^{14}C]glutamine was produced in 30 min.

Addition	Inhibition (%)		
	Observed	Cumulative	Additive
ADP (41) + AMP (37)	54	62	78
ADP (41) + PP_i (66)	79	79	107
ADP (41) + P_i (29)	62	58	70
AMP (37) + PP_i (66)	78	78	103
AMP (37) + P_i (29)	46	55	66
PP_i (66) + P_i (29)	67	75	95
AMP (37) + ADP (41) + PP_i (66)	83	87	144
AMP (37) + ADP (41) + P_i (29)	73	73	107
AMP (37) + ADP (41) + PP_i (66) + P_i (29)	86	90	173

adenine nucleotides, shown in Table III indicate that the inhibition by these metabolites was also cumulative.

Discussion

The present investigations show that as for glutamine synthetase from other organisms, the enzyme from *A. cylindrica* is also sensitive to various feedback inhibitors. Thus, the enzyme from this alga was inhibited by alanine, glycine, serine, aspartate, AMP and ADP. In addition, its activity was also depressed by PP_i and P_i, as was also reported for the enzyme from pea leaves [30]. Except for the adenine nucleotides, saturating concentrations of these metabolites resulted in a partial inhibition only of the enzyme activity. The cumulative inhibitory effects of the various combinations of amino acids, adenine nucleotides, PP_i and P_i implies that these modifiers act independently of each other. Similar patterns of inhibition have also been reported for glutamine synthetase from several other sources [17,18,30–33].

Of the various amino acids tested, alanine and glycine were most inhibitory, while aspartate and serine were less effective. When the four amino acids were present, each at 5 mM, they suppressed enzyme activity by 75%, so that they exert a profound regulatory influence on this enzyme. The inhibition of glutamine synthetase by alanine and glycine [17,20,34–37] and serine [30–32,38, 39] has also been observed in a variety of other organisms. Unlike the enzyme from *E. coli* [29,31] our results confirm the observation of Rowell et al. [20] that the enzyme from the blue-green alga is inhibited by aspartate. Woolfolk and Stadtman [31] have suggested that serine, an analogue of alanine, inhibits the enzyme by interacting at a relatively non-specific alanine-binding site. The differential effects of alanine and serine at varying concentrations of Mg^{2+} and their cumulative inhibition, reported herein, however, indicates that these amino acids interact with the enzyme by independent mechanisms. The inhibition of glutamine synthetase from *Bacillus stearothermophilus* by alanine but not serine [28] also supports the concept of separate binding sites for these amino acids.

Of the various nucleotides examined, enzyme activity was inhibited by ADP and AMP, but not by either CTP [15,28,32,35–37], GDP [28,36] or GTP [33,34] as shown for other organisms. The greater sensitivity of the algal enzyme to ADP than AMP is in accord with properties of the enzyme from other sources [30,31,36,37] but contrasts with the earlier reports [12,19] for a less purified enzyme from this alga. These differences are probably due to the presence of adenylate kinase in less purified enzyme preparations from the alga.

The activity of glutamine synthetase from *A. cylindrica* was affected markedly by small variations in the concentration of PP_i. Thus, on raising the concentration of PP_i from 2.0 to 2.4 mM its inhibitory effect increased from 10 to 50%. PP_i is a product of numerous ATP-utilizing reactions as in the biosynthesis of fatty acids, nucleotides, amino acids, polynucleotides, proteins and polysaccharides. It has been suggested that in some microorganisms biosynthesis of cellular constituents might be restricted because of a relatively slower rate of hydrolysis of PP_i [40]. The pronounced influence of PP_i, as reported herein, suggests that its elevated concentrations could have a significant regula-

tory effect on the activity of glutamine synthetase.

Although the inhibitory effects of various modifiers at limiting concentrations of ammonia have been studied, the precise mechanisms involved have not been determined. In *B. stearothermophilus* [38] alanine was less inhibitory at limiting concentrations of ammonia and Wedler et al. [28] reported a requirement for bound glutamate and ammonia to produce this effect. Our results indicate that the inhibition by the amino acids and adenine nucleotides became more pronounced at increasing, but still limiting, concentrations of ammonia. Thus it appears that with increasing concentrations of cellular ammonia, when the rate of the biosynthetic reaction is likely to be enhanced, the enzyme becomes increasingly more sensitive to the inhibition by the amino acids and adenine nucleotides.

The effects of ADP, AMP, PP_i and P_i on glutamine synthetase from the alga indicate that its activity, as proposed by earlier investigators for other organisms [19,30,36,37], is controlled by the energy status of the organism. ADP, AMP, P_i would be expected to accumulate whenever the ATP-generating reactions are impeded. Similarly, intracellular concentrations of PP_i should increase whenever the rate of ATP utilization exceeds the rate of PP_i hydrolysis. Thus, under conditions of declining intracellular ATP, these accumulated metabolites would restrain the utilization of ATP by glutamine synthetase in order to conserve this metabolite for biologically more important reactions. The enhanced inhibition of glutamine synthetase by the amino acids, adenine nucleotides and PP_i at higher concentrations of Mg^{2+} could also have a significant regulatory influence since the cellular concentration of free Mg^{2+} increases with diminishing adenylate charge [44]. The observed effect of Mg^{2+} on the inhibition by the modifiers of the algal enzyme would thus impart a greater sensitivity for regulating enzyme activity in relation to fluctuations in the energy charge of the cell. This enhanced response of the enzyme to these inhibitors at higher concentrations of Mg^{2+} may be due to an increased accessibility of binding sites to the modifiers because of the Mg^{2+}-induced conformational changes in the enzyme [32]. It is pertinent that Mg^{2+} had a profound effect on the inhibition by P_i of another ATP-utilizing enzyme, viz. ATP sulphurylase, in this alga [21]. It thus appears that changes in the concentration of Mg^{2+} play an important role in controlling ATP-utilizing reactions in this organism.

Acknowledgements

This work was supported by generous grants from the Australian Research Grants Committee and the Australian Water Resources Council. The skilled technical assistance of Mr. Michael Byrne is gratefully acknowledged.

References

1 Miflin, B.J. and Lea, P.J. (1976) Phytochemistry 15, 873—885
2 Nagatani, H., Shimizu, M. and Valentine, C.R. (1971) Arch. Microbiol. 79, 164—175
3 Brown, C.M., Mac-Donald, D.S. and Meers, M.J. (1974) in Advances in Microbial Physiology (Rose, A.H. and Tempest, D.W., eds.), Vol. 11, pp. 1—52, Academic Press, New York
4 Dharmawardene, M.W.N., Stewart, W.D.P. and Stanley, S.O. (1973) Planta, 108, 133—145
5 Stewart, W.D.P. and Rowell, P. (1975) Biochem. Biophys. Res. Commun. 65, 846—856

6 Wolk, C.P., Thomas, J., Shaffer, P.W., Austin, S.M. and Galonsky, A. (1976) J. Biol. Chem. 251, 5027—5034
7 Stewart, G.R. and Rhodes, D. (1976) FEBS Lett. 64, 296—299
8 Miflin, B.J. and Lea, P.J. (1977) Annu. Rev. Plant Physiol. 28, 299—329
9 Meister, A. (1962) in The Enzymes (Boyer, P.D., Lardy, H. and Myrbäck, K., eds.). 2nd edn., Vol. 6, pp. 247—266, Academic Press, New York
10 Stadtman, E.R. (1973) in Enzymes of Glutamine Metabolism (Prusiner, S. and Stadtman, E.R., eds.) pp. 1—6, Academic Press, New York
11 Woolfolk, C.A., Shapiro, B.M. and Stadtman, E.R. (1966) Arch. Biochem. Biophys. 116, 177—192
12 Dharmawardene, M.W.N., Haystead, A. and Stewart, W.D.P. (1973) Arch. Microbiol. 90, 281—295
13 Rhodes, D., Rendon, G.A. and Stewart, G.R. (1976) Planta 129, 203—210
14 Ginsburg, A. and Stadtman, E.R. (1973) in Enzymes of Glutamine Metabolism (Prusiner, S. and Stadtman, E.R., eds.), pp. 9—43, Academic Press, New York
15 Deuel, T.F. and Stadtman, E.R. (1970) J. Biol. Chem. 245, 5206—5213
16 Kingdon, H.S. (1974) Arch. Biochem. Biophys. 163, 429—431
17 Hubbard, J.S. and Stadtman, E.R. (1967) J. Bacteriol. 93, 1045—1055
18 Stadtman, E.R., Shapiro, B.M., Kingdon, H.S., Woolfolk, C.A. and Hubbard, J.S. (1968) in Advances in Enzyme Regulation (Weber, G., ed.), Vol. 6, pp. 257—289, Pergamon Press, London
19 Stewart, W.D.P., Haystead, A. and Dharmawardene, M.W.N. (1975) in Nitrogen Fixation by Free Living Microorganisms (Stewart, W.D.P. ed.), Vol. 6, pp. 129—158, Cambridge University Press, Cambridge
20 Rowell, P., Enticott, S. and Stewart, W.D.P. (1977) New Phytol. 79, 41—54
21 Sawhney, S.K. and Nicholas, D.J.D. (1977) Biochem. J. 164, 161—167
22 Shapiro, B.M. and Stadtman, E.R. (1970) Methods Enzymol. 17A, 910—922
23 Sawhney, S.K. and Nicholas, D.J.D. (1978) Planta, 139, 289—299
24 Tate, M.E. (1968) Anal. Biochem. 23, 141—149
25 Schwenn, J.D., Lilley, R.McC. and Walker, D.A. (1973) Biochim. Biophys. Acta, 325, 586—595
26 Wood, A.W., McCrea, M.E. and Seegmiller, J.E. (1972) Anal. Biochem. 48, 581—587
27 Lowry, O.H., Rosebrough, N.F., Farr, A.L. and Randall, A.J. (1951) J. Biol. Chem. 193, 265—275
28 Wedler, F.C., Carfi, J. and Ashour, A.E. (1976) Biochemistry 15, 1749—1755
29 Woolfolk, C.A. and Stadtman, E.R. (1964) Biochem. Biophys. Res. Commun. 17, 313—319
30 O'Neal, T.D. and Joy, K.W. (1975) Plant Physiol. 55, 968—974
31 Woolfolk, C.A. and Stadtman, E.R. (1967) Arch. Biochem. Biophys. 118, 736—755
32 Hachimori, A., Matsunaga, A., Shimizu, M., Samejima, T. and Nosoh, Y. (1974) Biochim. Biophys. Acta 350, 461—474
33 Kanamori, T. and Matsumoto, H. (1972) Arch. Biochem. Biophys. 152, 404—412
34 Kajinami, S. and Smith, E.C. (1971) J. Biochem. (Tokyo) 69, 429—432
35 Haystead, A. (1973) Planta 111, 271—274
36 Wedler, F.C. and Hoffman, F.M. (1974) Biochemistry 13, 3215—3220
37 McParland, R.H., Guevara, J.G., Becker, R.R. and Evans, H.J. (1976) Biochem. J. 153, 597—606
38 Hubbard, J.S. and Stadtman, E.R. (1967) J. Bacteriol. 94, 1016—1024
39 Tiemeler, D.C. and Milman, G. (1972) J. Biol. Chem. 247, 2272—2277
40 Klemme, J. (1976) Z. Naturforsch. 31c, 544—550
41 Blair, J.M. (1970) Eur. J. Biochem. 13, 384—390