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Regulation of glutamine synthetase activity

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Abstract

The role of glutamine as a donor of amino group and as a source of nitrogen for a variety of important precursors of macromolecules is discussed. The regulation of glutamine synthetase activity in *Escherichia coli* occurs by repression, cumulative feedback inhibition, by divalent cations, and by adenylylation and deadenylylation of the enzymes. The cascade consisting of the interaction of modifying enzymes and effectors, a novel method of regulation, was discovered using this enzyme system. The regulation in the case of Gram negative organisms is achieved by synergistic inhibition by end products and by simple product inhibition.

In view of the large concentration of glutamine present in the body fluids of several mammalian organisms, the regulation of glutamine synthetase does not appear to be of major significance.

Glutamine synthetase from plant sources has not been subjected to the same degree of intensive investigation. The enzymes from mung bean (*Phaseolus aureus*) was purified and shown to catalyze the 6-glutamyl transferase reaction by a ping-pong mechanism. Glycine, alanine and histidine as well as adenine nucleotides inhibited in a cumulative manner. Multiple inhibition analysis revealed non-exclusive binding sites for alanine and glycine and partial non-competitive and mixed type inhibition for these amino acids. Similar non-exclusivity in the binding sites for histidine and glycine were observed. ADP and AMP had mutually exclusive binding sites and were competitive inhibitors. The enzyme-antibody reaction was used to show that conformational changes occurred on the binding of substrates.

A comparison of the properties of the glutamine synthetases from bacterial, animal and plant sources revealed several common features and a few significant differences emphasizing the multitude functions of glutamine in the metabolism of the cell.

Key words : Regulation, glutamine synthetase.

1. Introduction

The ability to coordinate cellular activities to maintain homeostasis is one of the most remarkable features of a living organism. The coordinated control of a large number

of biosynthetic and degradative pathways is essential for an organism to adapt to changes in the internal and external environment. Since enzymes are critical components of various metabolic reactions, regulation of the activity of the enzymes provides a method for the delicate control of cellular processes. The control of the activity of enzymes of nitrogen metabolism is important as the nitrogenous compounds like proteins, nucleic acids, and complex polysaccharides, etc., are vital components of the cell. Since glutamine is the primary source of the nitrogen for a wide spectrum of metabolites, the enzymes of glutamine metabolism have been the targets of extensive investigation.

This brief review describes processes governing the regulation of glutamine synthetase activity and is divided into four sections. The first section discusses the role of glutamine in intermediary metabolism. The second deals with the control mechanisms of glutamine synthetase activity in microorganisms. The third section gives an account of the regulation of glutamine synthetase activity in mammals. The last section is a brief summary on the regulation of glutamine synthetase activity in plants.

2. Role of glutamine in intermediary metabolism

Glutamine participates *via* its α-amino group in the biosynthesis of amino acids[1]. In addition, the amide group of glutamine has a vital role in biosyntheses of tryptophan[2], histidine[3], glucosamine-6-phosphate, purine and pyrimidine nucleotides[1]. It is evident that glutamine is an intermediate in the ultimate synthesis of the major macromolecules of the cell, *viz.*, proteins, nucleic acids and complex polysaccharides. By virtue of its position in a highly branched metabolic pathway, it is obvious that glutamine synthetase activity should be under rigorous cellular control.

3. Regulatory processes of glutamine synthetase activity in microorganisms

Glutamine synthetase from *Escherichia coli* has been the most extensively studied and hence the discussion is mainly confined to this enzyme.

Glutamine synthetase catalyzes the synthesis of L-glutamine according to the following reaction :

$$\text{L-glutamate} + \text{ATP} + \text{NH}_4^+ \xrightarrow{\text{Me}^{2+}} \text{L-glutamine} + \text{ADP} + \text{P}_i. \qquad (1)$$

In addition to the biosynthetic reaction, the enzyme catalyzes several reactions which have no physiological significance[4]. However, these reactions are of interest for understanding the reaction mechanism. Extensive studies carried out on glutamine synthetase isolated from *E. coli*[5–9] have demonstrated that the enzyme can be regulated by four different mechanisms[4]. (*a*) Repression and derepression of enzyme synthesis in response to the variation in the concentration of ammonium salts; (*b*) Cumulative feedback inhibition by multiple end products of glutamine metabolism; (*c*) Interconversion between the

active (taut) and inactive (relaxed) forms of the enzyme in response to fluctuations in the divalent cation concentration; and (*d*) Cascade control by adenylylation and deadenylation reactions.

3.1. *Repression and derepression of glutamine synthetase*

Intracellular concentration of glutamine synthetase in *E. coli* varies markedly depending on the availability of carbon and nitrogen compounds in the growth medium[4]. As this review is concerned with the regulation of enzyme activity rather than enzyme synthesis, this aspect is not discussed.

3.2. *Cumulative feedback inhibition*

Woolfok and Stadtman[10] have reported that *E. coli* glutamine synthetase activity may be regulated through feedback inhibition by the multiple end products of glutamine metabolism. The fraction of the enzyme activity when mixtures of inhibitors are present is equal to the product of the fractional activities observed when each inhibitor is present individually. Cumulative feedback inhibition of the enzyme activity is also observed with glutamine synthetases isolated from other Gram-negative organisms such as *Psuedomonas fluorescens*, *Salmonella typhimurium* and *Micrococcus sadonesis*[11].

3.3. *Control of the enzyme activity by divalent cations*

Divalent cations are involved in the stabilization of the structure of glutamine synthetase[12, 13]. The native enzyme contains bound Mn^{2+} which can be removed by treatment with ethylenediaminetetracetate (EDTA) at pH 7·0 or by exhaustive dialysis. The sulphydryl groups of native enzyme, (referred to as the " taut " form) are inaccessible to alkylation suggesting that they are buried in the enzyme[14]. However, when Mn^{2+} is removed the enzyme is converted to a catalytically inactive (relaxed) configuration in which the sulphydryl groups are exposed. The " relaxed " form is converted to a catalytically active form referred to as the " tightened " form, by the addition of divalent cations (either Mg^{2+}, Ca^{2+} or Mn^{2+}). The role of metal ions in the interconversion of " relaxed " and " taut " forms suggests that they participate in the regulation of glutamine synthetase activity. Fluctuations in the divalent cation concentrations can occur by changes in the cellular concentrations of metal chelators such as nucleoside pyrophosphates, citrate, etc.[4].

3.4. *Cascade control by adenylylation and deadenylylation reactions*

Adenylylation of glutamine synthetase involves a covalent modification of the enzyme through the incorporation of AMP attached by a phosphodiester linkage to the hydroxyl group of a specific tyrosyl residue of the enzyme. Adenylylation of *E. coli* glutamine synthetase occurs when the cells are grown on a medium containing low concentrations of NH_4^+ [17].

Deadenylylation of the adenylylated glutamine synthetase involves a phosphorolytic cleavage of the adenylyltrosyl bonds to form ADP and the deadenylylated enzyme. Deadenylylation of the adenylylated enzyme occurs in response to low concentrations of NH_4^+ in the growth medium of *E. coli* cells[20, 21]. The physiological significance of adenylylation and deadenylylation is apparent from the fact that the relative intracellular concentrations of the adenylylated and deadenylylated forms of glutamine synthetase vary widely in response to availability of nitrogen in the medium.

The adenylylation and deadenylylation of glutamine synthetase are catalyzed by adenylyltransferase[20, 21] according to the following reactions :

$$12\ ATP + \text{glutamine synthetase} \xrightarrow[\text{transferase, } Mg^{2+}]{\text{adenylyl}} \text{glutamine synthetase } (AMP)_{12} + 12\ PP_i \quad (2)$$

$$\text{Glutamine synthetase } (AMP)_{12} + 12\ PP_i \xrightarrow[\text{transferase, } Mg^{2+}]{\text{adenylyl}} \text{glutamine synthetase} + 12\ ADP \quad (3)$$

The coupling between the adenylylation and deadenylylation activities is prevented by the action of an elaborate cascade system comprising of metabolite regulated enzymes[1, 4]. In the absence of such a regulation a futile cycle exists in which glutamine synthetase fluctuates between the unadenylylated and the adenylylated forms and ATP undergoes phosphorolysis to ADP and pyrophosphate as shown in eqn. (4).

$$12\ ATP + 12\ P_i \longrightarrow 12\ ADP + 12\ PP_i \quad (4)$$

The cascade consists of three metabolite regulated enzymes and a small regulatory protein all of which together modulate adenylylation and deadenylylation of glutamine synthetase and thereby determine its catalytic potential, susceptibility of feedback inhibition and divalent cation specificity[1]. The proteins that function in the cascade are (i) regulatory protein; (ii) uridylyltransferase which catalyzes uridylylation of the regulatory protein; (iii) uridylyl removing enzyme (UR-enzyme) catalyzing cleavage of 5′-UMP from the regulatory protein; and (iv) adenylyltransferase catalyzing the adenylylation and deadenylylation of glutamine synthetase in the presence of deuridylylated and uridylylated regulatory protein, respectively.

Basically, the regulation is achieved by two interconverting enzyme systems that are under metabolite control. The average state of adenylylation of glutamine synthetase is determined by the ratio of deuridylylated regulatory protein (P_{IIA}) to uridylylated regulatory protein (P_{IIUMP}). This ratio is, in turn, governed by the activities of uridylyltransferase and UR enzyme, which are dependent on the intracellular concentrations of UTP, ATP, α-ketoglutarate, glutamine and P_i. α-Ketoglutarate and ATP inhibit the P_{IIA}

stimulated adenylylation activity of adenylyltransferase. They are required for both uridylylation activity of uridylyltransferase and for P_{IIUMP} stimulated deadenylylation of glutamine synthetase. On the contrary, glutamine and P_i stimulate the P_{IIA} enhanced adenylylation activity of adenylyltransferase. According to Mangum *et al*[22] the teleologic significance of this observation is as follows. A high glutamine : α-ketoglutarate ratio favours adenylylation (inactivation) of glutamine synthetase; whereas, a low ratio favours deadenylylation (activation) of the enzyme. Since α-ketoglutarate is a precursor of glutamine, concentration of these metabolites varies inversely with respect to each other depending on the availability of ammonium nitrogen.

3.5. *Properties of adenylylated and unadenylylated glutamine synthetase*

Although adenylylation does not result in alteration in many of the physical and chemical properties, it produces several marked changes in the regulatory and catalytic properties[23–25]. On adenylylation, the divalent cation specificity changes from Mg^{2+} for the biosynthetic activity of the enzyme. Compared to the unadenylylated enzyme, the biosynthetic and the transferase activities of the adenylylated enzyme are more sensitive to feedback inhibition by histidine, tryptophan, CTP and AMP[26, 27].

In conclusion, the myriad effects of covalent modification, feedback inhibition, and metal ions on glutamine synthetase, harmoniously regulate the availability of several key metabolites for the growth and maintenance of the Gram-negative bacteria.

4. Regulation of the activity of glutamine synthetases isolated from Gram-positive organisms

Glutamine synthetases from *Bacillus subtilis*[28,30], *B. licheniformis*[31,32] and *B. stearothermophilus*[33–35] have been extensively studied. As in the case of Gram-negative organisms, the enzymes isolated from Gram-positive organisms are also subject to feedback inhibition by the end products of glutamine metabolism. However, the inhibitors act synergistically, and product inhibition by glutamine is a key feature of enzyme regulation[11].

4.1. *Glutamine synthetase of* B. subtilis

The physical characteristics of the enzyme are very similar to those of the *E. coli* glutamine synthetase. The biosynthetic activity of the enzyme is activated by both Mn^{2+} and Mg^{2+}. The enzyme is subject to feedback inhibition by the end products of glutamine metabolism. The kinetics and the sensitivity to feedback inhibition by each of these products are dependent upon whether Mg^{2+} or Mn^{2+} is used as the activating cation. This intrinsic property of the enzyme is not dependent on the growth conditions of the bacterial cells.

In the Mn^{2+}-dependent biosynthetic assay, the inhibition by glutamine is greatly potentiated by AMP. Histidine and AMP are also synergistic, but glutamine acts independent

of histidine. In the Mg^{2+}-dependent biosynthetic assay, alanine and glycine produce no effect, but these amino acids are strong inhibitors in the Mn^{2+}-dependent reactions.

In *B. subtilis*, glutamine plays a major role in the overall regulation of its own synthesis. In *E. coli*, glutamine modulates adenylylation and deadenylylation processes, and thereby regulates the overall enzyme activity and responsiveness to feedback inhibitors. In *B. subtilis*, similar regulatory effects are achieved by the direct inhibition of the enzyme activity by glutamine and by the striking potentiation of AMP inhibition at low concentrations of glutamine. Therefore, the intracellular levels of glutamine play an important role in the delicately balanced control of *B. subtilis* glutamine synthetase leading to maintenance of nitrogen balance in the cell[36].

4.2. *Glutamine synthetase of* B. stearothermophilus

The enzyme has been purified to homogeneity and is shown to be feedback regulated by AMP, ADP, GDP, CTP, glycine, alanine, histidine, tryptophan, carbamyl phosphate and glucosamine-6-phosphate. Many of these inhibitors are partial inhibitors of the enzyme activity. The enzyme is synergistically inhibited by AMP in the presence of glutamine, histidine, ADP or glucosamine-6-phosphate. Synergistic inhibition of the enzyme activity is also noticed for CTP with ADP or GDP. In addition, the enzyme activity is also subject to cumulative feedback inhibition by some of these metabolites.

4.3. *Glutamine synthetase of* B. licheniformis

Hubbard and Stadtman[32], using the partially purified enzyme from *B. licheniformis*, have demonstrated that the enzyme is feedback inhibited by glutamine, AMP, histidine, ADP, alanine and glycine. These act synergistically towards glutamine inhibition. Alanine also acts antagonistically towards glycine and histidine inhibition. Cumulative feedback inhibition is observed with histidine + glycine, glycine + AMP, AMP + alanine, indicating that these metabolites act independent of each other. In conclusion, the mechanisms of regulation of glutamine synthetase activity in microorganisms are elaborate and elegant for a coordinated functioning of cellular activities.

5. Regulation of glutamine synthetase activity in mammals

Glutamine is the most abundant amino acid constituent of mammalian blood and tissues. The regulation of glutamine synthesis in mammalian tissues may be of trivial significance (in terms of glutamine requirement for various biosynthetic reactions), in view of the large tissue concentration. The enzyme has been isolated from rat, ovine, porcine, human brain and sheep spleen[37–44] and rat and Chinese hamster liver[42, 45–47].

A few attempts have been made to speculate on the possible regulatory mechanisms. The activity of the ovine brain glutamine synthetase is affected by relative concentrations of various nucleotides and metal ions[48]. The rat liver glutamine synthetase resembles

ovine brain glutamine synthetase in its subunit composition and molecular weight[46]. The enzyme is activated[42] by α-ketoglutarate in the presence of Mg^{2+}, or Mn^{2+}. Histidine and the reaction product, glutamine, are also inhibitors of the catalytic activity. The enzyme becomes sensitive to inhibition by alanine, glycine, serine and CTP only when Mn^{2+} is present in the reaction mixture. In this context, it is pertinent to note that the adenylylated glutamine synthetase, which requires the presence of Mn^{2+} for its biosynthetic activity, is more susceptible to feedback inhibitors than the deadenylylated enzyme.

The activation of the enzyme by α-ketoglutarate provides a mechanism of feed-forward activation, whereby the precursor, α-ketoglutarate (produced by citric acid cycle and transamination reactions) can stimulate glutamine formation. It is interesting to recall the effect of α-ketoglutarate in increasing the glutamine formation indirectly by mediating the stimulation of deadenylylation of glutamine synthetase and the inhibition of adenylylation of *E. coli* glutamine synthetase to produce a more active enzyme[22].

6. Glutamine synthetase of plants

Compared to the vast amount of information on the glutamine synthetases of mammals and bacteria, very little is known on the enzyme from plants. The enzyme has been isolated from pea leaves[49–51], pea seeds[52], soyabean root nodules[53], rice plant roots[54] and mung bean[55]. In leguminous plants, glutamine is one of the early products of the ammonia assimilation process[56, 57] and an important component transported from the nodules to the plant[58].

Glutamine synthetase is believed to play a key role in the assimilation, storage and translocation of ammonia in higher plants[49, 59]. Furthermore, it is possible that glutamine synthetase, which plays a key role in nitrogen metabolism, may be important in the germination of seeds and growth of plants.

The pea leaf glutamine synthetase has been purified to homogeneity by O'Neal and Joy[49–51]. The enzyme is significantly inhibited by ADP and AMP in the Mg^{2+}-dependent biosynthetic assay. Among the amino acids, histidine and ornithine are the most inhibitory, but significant inhibition is observed only in the presence of Mn^{2+}. Alanine, glycine and serine also cause slight inhibition of the (Mn^{2+}-dependent activity) enzyme activity. Cumulative inhibition of the enzyme activity is observed in the presence of these amino acids.

The partially purified enzyme from rice plant roots is regulated by the end products of glutamine metabolism. The nucleotides, AMP, ADP, GTP and IDP cause substantial inhibition of the enzyme activity. The combined effects of these nucleotides result in cumulative inhibition of the enzyme activity. The homogeneous enzyme isolated from soyabean root nodules is feedback-regulated by GTP, ADP, AMP, histidine and glycine.

6.1. *Mung bean glutamine synthetase*

The enzyme from the germinated seedlings of mung bean is purified to homogeneity. The molecular weight of 775,000 ± 25,000 for mung bean glutamine synthetase is higher than that reported for this enzyme from other sources. The subunit molecular weight of mung bean glutamine synthetase as determined by SDS-gel electrophoresis is 50,000. As the molecular weight of mung bean enzyme was determined by only one method of moderate accuracy, the data cannot be employed to calculate with confidence the number of subunits in this plant enzyme. The precise determination of the number of subunits in mung bean glutamine synthetase is possible only after an accurate estimation of molecular weight by several methods.

The bisubstrate kinetics of the γ-glutamyl transferase reaction (shown below) catalyzed by

$$\text{Glutamine} + \text{hydroxylamine} \xrightarrow[P_i \text{ or arsenate}]{Me^{2+},\ ADP} \gamma\text{-glutamylhydroxate} + NH_4^+ \qquad (5)$$

This enzyme revealed that the catalysis proceeds by a ping pong mechanism. In reciprocal plots, parallel set of lines are obtained when both glutamine and hydroxylamine are the variable substrates in the presence of different concentration of the other substrate. In the product inhibition studies, glutamine and γ-glutamylhydroxamate pair, and hydroxylamine and ammonia pair give competitive inhibition; glutamine and ammonia pair, or hydroxylamine and γ-glutamylhydroxamate pair gives noncompetitive inhibition, and, in each case the product inhibition is eliminated by saturation with the non-varied substrate. This steady-state kinetics of γ-glutamyltransferase reaction is consistent with a ping pong mechanism. This mechanism predicts the formation of γ-glutamyl enzyme intermediate, which needs to be confirmed.

The amino acids, alanine, glycine and histidine partially inhibit the biosynthetic activity of the enzyme. Alanine is a partial noncompetitive, histidine, a partial competitive and glycine, a partial mixed type inhibitor when glutamate is the varied substrate. The partial inhibition can be fitted into the models of Stadtman *et al*[7] and Segel[60]. In the model of Stadtman *et al*[7], the enzyme possesses two distinct and mutually exclusive inhibitor sites. When the inhibitor binds to one of the sites (the " inhibitory " site) it induces a conformational change in the enzyme that either prevents the substrate from binding to the catalytic site or prevents the catalytic activity even though the substrate is bound to the enzyme. The binding of the inhibitor to the other inhibitor site (the " noninhibitory site ") is also prevented. When the inhibitor binds to the noninhibitory site, binding of the inhibitor to the inhibitory site is prevented but there is no effect on catalytic activity of the enzyme. The following equilibria explain the partial competitive inhibition by histidine [Fig. 1 (*a*)] and partial noncompetitive and mixed type inhibition by alanine and glycine [Fig. 1 (*b*)], respectively. Although the data on partial inhibition can be adequately explained by these equilibria, alternate explanation cannot be ruled out and is described below.

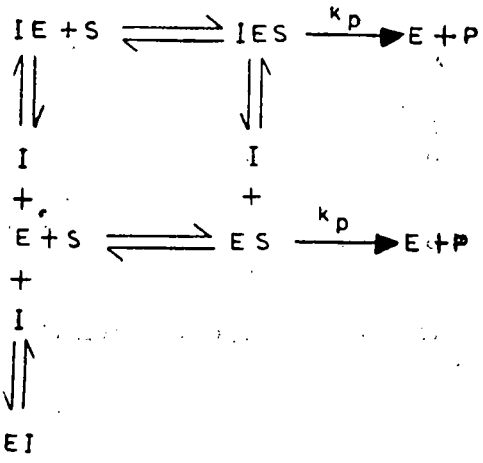

FIG. 1 (*a*). I—Histidine; EI—histidine bound to the inhibitory site; IE—histidine bound to the non-inhibitory site; E—enzyme in the presence of saturating concentrations of ATP and hydroxylamine.

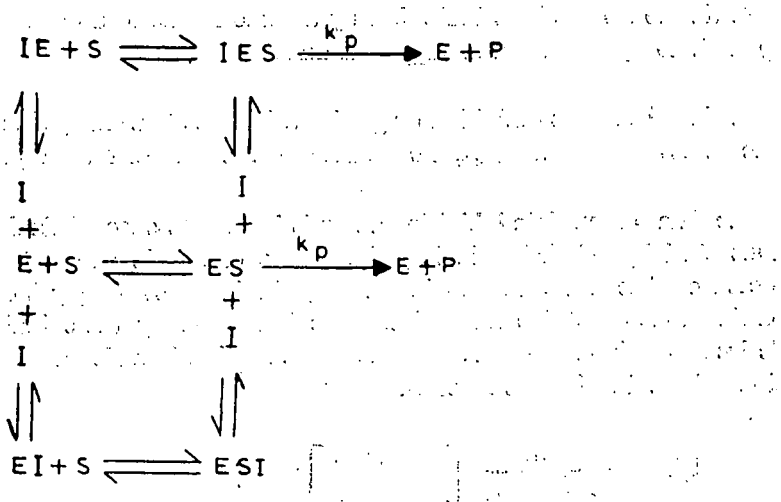

FIG. 1 (*b*). I—Alanine or glycine; EI—inhibitor bound to the inhibitory site; IE—inhibitor bound to the non-inhibitory site; E—enzyme in the presence of saturating concentrations of ATP and hydroxylamine.

In the model of Segel[60], the inhibitors bind to their single specific sites to yield enzyme-inhibitor and enzyme-substrate-inhibitor complexes. The enzyme-substrate-inhibitor complex can yield products with equal or less facility than the enzyme-substrate complex. The partial competitive inhibition by histidine, noncompetitive inhibition by alanine and mixed type inhibition by glycine can be explained using the equilibria shown in Fig. 2.

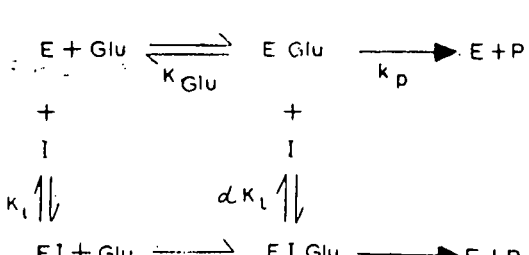

FIG. 2. E—Enzyme in the presence of saturating concentrations of ATP and hydroxylamine; I—histidine, alanine or glycine.

Partial competitive inhibition by histidine is explained by the value of $\alpha = 2{\cdot}4$ and $K_{His} = 100$ mM in this case $kp \propto kp$, as both ES and ESI complexes are equally efficient in producing the products.

Partial noncompetitive inhibition by alanine is due to a decrease in the rate constant by a factor $\beta = 0{\cdot}53$ and $K_{ala} = 21$ mM.

Partial mixed type inhibition by glycine arises due to a change in both the V_{max} and K_{Glu}. The values of α, β and K_{Gly} are 3·014, 0·137 and 22·5 mM respectively.

The data on multiple inhibition analysis in the presence of alanine and glycine suggest nonexclusivity of their binding sites. The equilibria shown in Fig. 3 (*a*) explain the presence of separate and noninteracting binding sites for glycine and alanine, and the partial noncompetitive and mixed type inhibition by these inhibitors. The equation derived for Dixon plot when glycine concentration is varied at different fixed concentration of alanine is given below.

$$1/V = \frac{K_{Ala}}{[Glu]\,V\,K_{Gly}}\left[1 + \frac{[Ala]}{K_{Ala}}\right][Gly]$$

$$+ 1/V\left[1 + \frac{K_{Glu}}{[Glu]} + \frac{[Ala]}{K_{Ala}} + \frac{[Gly]}{K_{Gly}}\right.$$

$$+ \frac{[Gly]\,[Ala]}{K_{Gly}\,K_{Ala}} + \frac{[Glu]\,[Ala]}{K_{Glu}\,K_{His}} + \frac{[Gly]\,[Glu]}{\alpha\,K_{Gly}\,K_{Glu}}$$

$$\left. + \left[\frac{[Glu]\,[Ala]\,[Gly]}{\alpha\,K_{Glu}\,K_{Ala}\,K_{Gly}}\right]\right] \qquad (6)$$

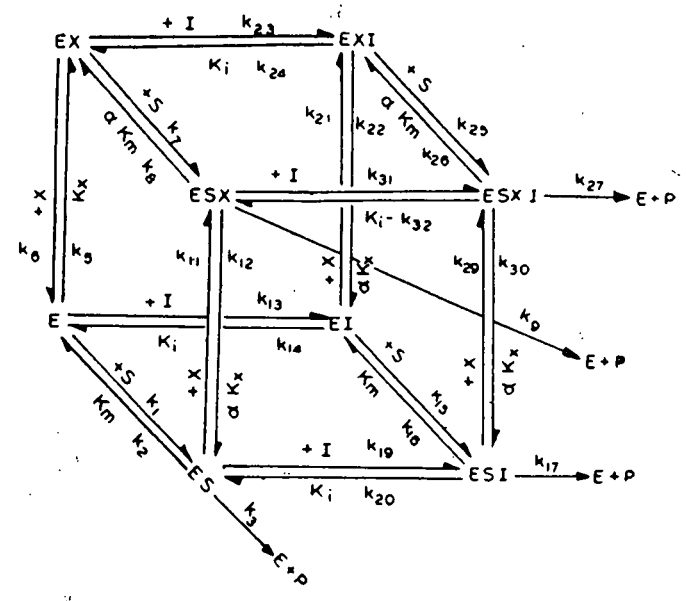

FIG. 3 (*a*). Equilibria among enzyme-species in the presence of a noncompetitive inhibitor (I) and a mixed-type inhibitor (X) (156).

As the concentrations of glycine and alanine used in these experiments were not very much larger than their K_i values, the concentrations of E. Gly. Glu, E. Glu. Ala. Glu were assumed to be low and may not, therefore, contribute significantly to the velocity. These terms have not been considered in the numerator. Under these conditions the distribution of free enzyme among the various enzyme-inhibitor complexes is sufficiently large to reduce the velocity. The validity of these assumptions is established by the linear Dixon Plot obtained when glycine concentration is varied in the presence of different fixed concentrations of alanine. The nonexclusivity of the binding sites for histidine and glycine is explained by the equilibria shown in Fig. 3 (*b*). The equation for Dixon Plot derived using the same assumptions as above is given on page 13.

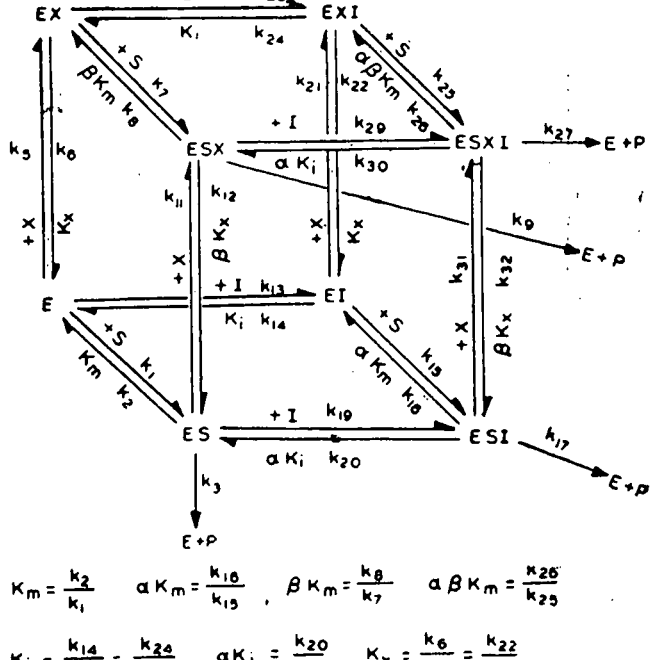

FIG. 3 (*b*). Equilibria among enzyme forms in the presence of a competitive inhibitor (I) and a mixed-type inhibitor x.

In addition to amino acids, glutamine synthetase activity is shown to be regulated by ADP and AMP. These two nucleotides are mutually exclusive competitive inhibitors for ATP as explained by the equilibria (Fig. 4). Cumulative inhibition observed with alanine, glycine and histidine support the contention that these amino acids possess separate and nonexclusive binding sites. This also suggests that the binding of one effector has no effect on the binding of the other. Cumulative inhibition is also observed when the amino acids interacted with AMP and ADP indicating that the binding sites for these nucleotides are different from the amino acid binding sites[62].

The results presented on mung bean glutamine synthetase indicate that adenine nucleotides and amino acids regulate the activity of the enzyme and the enzyme possesses

$$1/v = \frac{K_{Glu}}{[Glu]\,V\,K_{Gly}}\left[1 + \frac{[His]}{K_{His}}\right][Gly]$$

$$+ 1/V\left[1 + \frac{K_{Glu}}{[Glu]} + \frac{[His]}{K_{His}} + \frac{[Gly]}{K_{Gly}}\right.$$

$$+ \frac{[Gly][His]}{K_{Gly}K_{His}} + \frac{[Glu][His]}{\alpha K_{Glu}K_{His}}$$

$$\left. + \frac{[Gly][Glu]}{\beta K_{Gly}K_{Glu}} + \frac{[Glu][His][Gly]}{\alpha\beta K_{Glu}K_{Ala}K_{Gly}}\right] \qquad \text{Eq. 7}$$

separate sites for the regulators. The existence of separate binding sites for the effectors needs to be confirmed by differential inactivation of one of these sites, combined with kinetic studies, and by binding and isotope exchange studies.

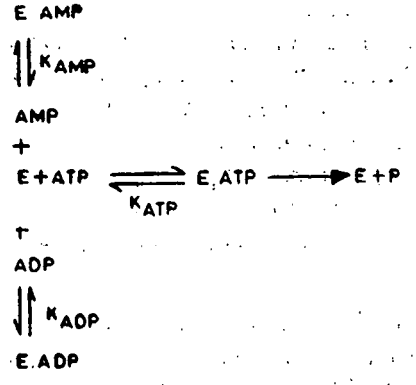

FIG. 4.

Mung bean glutamine synthetase activity is completely inhibited by its antibody. Preincubation of the enzyme with glutamate and ATP, prior to the addition of antibody, partially protects the enzyme against inhibition. This complex is referred to as the protected enzyme-antibody complex. The K_m values of this complex for the substrates are unaltered from those of the native enzyme. The K_m values of the partially inhibited enzyme (the enzyme pretreated with the antibody prior to the addition of substrates) are two-fold higher than those of the native enzyme. These results indicate that the

substrate induced conformational changes in the enzyme are responsible for the protection of the enzyme activity against inhibition by its antibody[63].

The properties of mung bean glutamine synthetase are compared in Table I, with those of the enzyme isolated from *E. coli*, *B. subtilis*, *B. stearothermophilus*, rat liver, sheep brain, pea leaf, rice plant and soyabean. The molecular weight of the mung bean enzyme was higher than that reported from other sources. The subunit molecular weight of mung bean glutamine synthetase as determined by SDS-gel electrophoresis was 50,000; a similar value was obtained for this enzyme from other sources. The mung bean enzyme is apparently less stable than the enzyme from other sources. Addition of ADP, EDTA and 2-mercaptoethanol stabilized the enzyme. In analogy with the role of AMP in the regulation of *E. coli* glutamine synthetase, it is interesting to speculate a similar role of ADP in the stabilization and regulation of glutamine synthetase from this plant source.

Our results suggest that the γ-glutamyl transferase reaction catalyzed by the mung bean glutamine synthetase proceeds by a ping-pong mechanism with glutamine as the leading substrate and γ-glutamylhydroxamate as the last product to be released from the enzyme[61]. This mechanism predicts the formation of a γ-glutamyl intermediate. It can be recalled that the biosynthetic reaction of the sheep brain and pea seed glutamine synthetase was postulated to involve the formation of a stable intermediate, γ-glutamyl phosphate. Considerable evidence exists both in support and against the possible existence of this covalent intermediate in the pathway of the enzyme reaction. Rhee *et al*[64] are of the opinion that in analogy with glutamine synthetase activity, the γ-glutamyl transferase reaction also proceeds by either a concerted or stepwise mechanism with the formation of γ-glutamyl phosphate or arseniate. In a similar manner if a single catalytic site is involved in the transferase and synthetase reactions, it could be predicted that a γ-glutamyl enzyme intermediate (probably glutamyl phosphate) is formed in the biosynthetic reaction catalyzed by the mung bean enzyme.

The high concentrations of amino acid and nucleotide effectors required to cause significant inhibition makes it difficult to interpret their *in vivo* importance in the regulation of this enzyme activity. The internal pH of the plant cells may be different from that used in the *in vitro* experiments and this could affect the interaction of the enzyme with the effectors. The metal ion present and pH profoundly regulate the activity of the soyabean enzyme[53]. The cumulative nature of the inhibition could result in significant decrease in enzyme activity when all of them are present in moderate amounts. It is pertinent to emphasise that the extensively studied glutamine synthetase of *E. coli*[7] and other microorganisms[11, 31, 35], as well as the mammalian enzyme[42], the individual effectors must be present in millimolar concentrations for significant inhibition. It must be pointed out that even though the inhibition is low at high concentration of substrates, at K_m values of glutamate significant inhibition was noticed. Pea leaf glutamine

Table I

Comparison of the properties of glutamine synthetase

	E. coli	*B. subtilis*	*B. stearo-thermo-philus*	Rat liver	Sheep brain	Pea leaf	Rice plant	Soya-bean	Mung bean
Molecular weight	600000	600000	600000	352000	392000	370000	..	376000	775000
Subunits (MW)	50000	50000	51000	44000	49000	..	..	47300	50000
Number	12	12	12	8	8	..	..	8	
K_m (mM)									
L-Glutamate	2·4	0·8	1·5	5·0	2·5	9·3	3·75	..	2·5
ATP	0·68	0·2	0·3	..	2·3	0·43	0·44	..	1
NH_3 or NH_3OH	1·8	0·4	1·0	..	0·18	0·84	0·4	..	0·5
Inhibited by :	Ala	Ala	Ala	Ala	Ala	Ala	..	..	Ala
	His	His	His	..	..	..	..	His	His
	Gly	Gly	Gly	Gly	Gly	Gly	..	Gly	Gly
	Try	Try	..	..	..	..	..	..	..
	CP	..	CP	CP	CP	..	..	..	..
	Glcn-6-P	..	Glcn-6-P	..	..	..	..	..	..
	AMP	AMP	AMP	..	..	AMP	AMP	AMP	AMP
	CTP	..	CTP	CTP	..	..	..	CTP	..
	..	..	ADP	..	..	ADP	ADP	ADP	ADP
	..	..	GDP	..	..	..	..	..	..
	..	..	..	..	..	..	GTP	..	..
	..	..	..	..	..	..	IDP	..	..
	..	L-Gln	L-Gln	..	..	..	..	..	..
Regulation by covalent modification	Yes	No	No	No	No	No	No	No	No
Cumulative feedback inhibition	Yes	No	Yes	No	No	Yes	No	No	Yes
Synergistic inhibition	No	Yes	Yes	No	No	No	No	No	No

synthetase was also inhibited in a cumulative manner by histidine, ornithine, alanine, glycine and serine.

Multiple inhibition analysis suggested separate binding sites for the amino acid inhibitors[62]. On the contrary, similar studies with glutamine synthetase showed that alanine and glycine share a common binding site. The inhibition of nucleotides is a common feature of several glutamine synthetases[10, 35, 50, 51, 54]. Unlike these enzymes, the mung bean and pea leaf enzymes are not inhibited by nucleotides other than AMP and ADP.

The discussion presented in this review illustrates that significant variations exist in the mechanisms of regulation of the activity of glutamine synthetases isolated from different sources. These variations reflect the multitude of functions of glutamine in the metabolism of the cell.

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