Regulation of Glutamine Synthetase Synthesis in some Gram-Negative Bacteria

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(Received 2 July 1970)

Although most of the bacteria that can synthesize glutamine can be shown to possess the enzyme glutamine synthetase [γ -glutamate-ammonia ligase (ADP), EC 6.3.1.2], the activity and/or cellular content of this enzyme may vary markedly with the growth conditions (Woolfolk, Shapiro & Stadtman, 1966; Meister, 1968). The nature of the mechanisms regulating glutamine synthetase synthesis, particularly in *Escherichia coli*, have been studied extensively (Woolfolk *et al.* 1966; Wu & Yuan, 1968; Pateman, 1969), and, on the basis mainly of batch-culture experiments (in which the nitrogen source in the medium was varied quantitatively and qualitatively), the central role of ammonia in the regulatory process has been recognized. However, it has not been possible to decide from these experiments whether ammonia itself represses glutamine synthetase synthesis or whether some other metabolite formed from ammonia (for example, glutamine) is the active substance. The situation is confused by the fact that many amino acids (glutamine, asparagine, arginine, methionine, tryptophan), when added to the growth medium, suppress the synthesis of glutamine synthetase; and one amino acid (L-glutamate) seemingly can act as an inducer (Wu & Yuan, 1968).

In each of the above-mentioned studies, no distinction was drawn between the concentrations of substances initially added to the environment and their concentrations intracellularly during growth and enzyme synthesis; indeed, invariably it was assumed that the compositions and contents of bacterial 'pools' mirrored exactly the compositions of their growth environments. This is unlikely to have been true, and, since only metabolites actually present within the bacterial cell are in a position to react with the mechanisms controlling enzyme synthesis, it would have been more germane to have related differences in enzyme content, not to differences in the chemical environment, but to differences in pool composition and content. We have now done this and thereby resolved some of the conflicting statements about the roles of specific metabolites (ammonia, glutamine, glutamate) in the induction and repression of synthesis of this enzyme. This paper presents

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evidence that supports the hypothesis that, in several Gram-negative bacteria at least, ammonia (and only ammonia) is the repressor of glutamine synthetase synthesis.

It is difficult to establish quantitative relationships between the chemical environment and the cellular content of a particular enzyme when organisms are growing in a batch-type culture, since both may vary continuously with time. However, with cultures growing in a chemostat these problems do not arise, since, once the culture is in a steady state, the environment does not change with time and neither, it is reasonable to suppose, does the bacterial content of most enzymes. Further, specific environmental conditions can be established in a chemostat culture in which the extracellular and intracellular concentrations of particular metabolites can be maintained at desired values. Therefore we have used a chemostat to obtain conditions in which the intracellular concentrations of ammonia, glutamine and glutamate varied separately and have correlated these changes with changes in the bacterial content of glutamine synthetase.

Cultures of *Aerobacter aerogenes* (N.C.T.C. 418), *Erwinia carotovora* (M.R.E. 604), *Pseudomonas fluorescens* (KB-1) and *Escherichia coli* (M.R.E. 162) were grown in 500 ml 'Porton-type' chemostats (Herbert, Phipps & Tempest, 1965) at a dilution rate of 0.3 h^{-1} , with the temperature maintained at 35°C and the pH value regulated at 6.8. The defined simple salts media were as described by Evans, Herbert & Tempest (1970), except that in some cases (where indicated) the sole utilizable nitrogen source was glutamate instead of ammonia. Bulk samples (0.5–1.0 litre, containing 2–4 g wet wt. of bacteria), required for the extraction of glutamine synthetase (by the method of Hughes, 1951) and its assay (by the method of Elliott, 1955), were obtained by collecting into an ice-cooled receiver the culture flowing from the chemostat vessel. Smaller samples (20–30 ml), required for the extraction of the free amino acid pool (by the method of Tempest, Meers & Brown, 1970b) and for determination of the culture bacterial dry weight (by the method of Tempest, Hunter & Sykes, 1965), were drawn directly from the culture vessel.

When cultures of the Gram-negative bacteria

Table 1. *Glutamine synthetase activities in some Gram-negative bacteria that were grown, in chemostats, in media containing either an excess, or growth-limiting concentration, of the nitrogen source (ammonia or glutamate)*

The cultures were grown in 500 ml 'Porton-type' chemostats (dilution rate 0.3 h^{-1} ; 35°C ; pH 6.8) in simple salts media in which the sole utilizable nitrogen source was either NH_3 or glutamate. Extracts for the assay of 'pool' constituents were obtained by treating concentrated suspensions of organisms with ice-cold 0.25 M-HClO_4 (for 10 min) and separating the supernatant fluid after centrifugation ($5000g$ for 10 min). Cell-free extracts for enzyme assay were prepared by disrupting suspensions of organisms in a Hughes (1951) press, dispersing the crushed organisms in 0.05 M-tris-HCl buffer, pH 7.8, and removing the cell debris by centrifugation ($15000g$ for 30 min). Glutamine synthetase activity of each extract was assayed, at pH 7.8, by the method of Elliott (1955).

Organism	Growth condition	'Pool' concentrations (mM)			Glutamine synthetase activity ($E_{520}^{1.2\text{cm}}$ /h per mg of protein)
		NH_3	Glutamate	Glutamine	
<i>A. aerogenes</i>	Glucose-limited	>10	3.4	<0.1	0.1
	Glucose-limited + 2% NaCl	>10	37.4	1.3	0.1
	NH_3 -limited	1.0	5.8	0.1	1.6
	NH_3 -limited + 2% NaCl	1.2	30.2	0.4	1.7
	* N-source (glutamate)-limited	0.7	5.0	0.2	2.8
	* Phosphate-limited (N source: glutamate)	>10	>20.0	4.0	0.2
<i>Er. carotovora</i>	Glucose-limited	>10	5.5	0.4	0.2
	NH_3 -limited	2.9	4.7	0.4	1.7
	* N-source (glutamate)-limited	2.0	4.6	0.4	3.0
	* Phosphate-limited (N source: glutamate)	>10	>30	8.0	0.4
<i>Ps. fluorescens</i>	Glucose-limited	>10	4.0	0.1	0.1
	NH_3 -limited	1.8	9.6	1.4	4.3
<i>Esch. coli</i>	Glucose-limited	>10	3.0	<0.1	0.1
	NH_3 -limited	1.3	6.1	0.2	1.4

* In these cultures glutamate provided the sole source of utilizable nitrogen.

listed above were grown in environments that contained an excess of the nitrogen source (e.g. were glucose-limited or phosphate-limited), the pool ammonia concentration was invariably greater than 10 mM and the glutamine synthetase activity of the organisms was low (Table 1). Thus, irrespective of whether the nitrogen source was free ammonia or glutamate, glutamine synthetase synthesis was repressed. Conversely, when these bacteria were grown in environments that lacked an excess of the nitrogen source [e.g. were nitrogen-source (glutamate)-limited or ammonia-limited], the pool ammonia concentration was low and the glutamine synthetase activity was substantial. The glutamine synthetase content in nitrogen-limited *A. aerogenes* and *Er. carotovora* organisms was greater when nitrogen was supplied as glutamate than when it was supplied as ammonia (Table 1). This could be taken to support the hypothesis (Wu & Yuan, 1968) that glutamate induces the synthesis of this enzyme; however, it is clear from Table 1 that, when the utilizable nitrogen source was glutamate and nitrogen availability limited growth, the pool ammonia concentration was even lower than when the same organisms were ammonia-

limited. Presumably the glutamate nitrogen could be utilized directly for cell synthesis without being cycled through ammonia; thus the ammonia concentration in these bacterial pools was even further decreased and the synthesis of glutamine synthetase even less repressed than when ammonia availability limited growth.

A further indication that glutamate does not induce glutamine synthetase synthesis follows from the observation that addition of sodium chloride (2%, w/v) to glucose-limited or ammonia-limited chemostat cultures of *A. aerogenes* greatly increased the pool free glutamate concentrations (see Table 1 and Tempest *et al.* 1970b), but had little effect, if any, on their content of glutamine synthetase. With cultures of *Er. carotovora* there was again no correlation between the cellular contents of glutamate and glutamine synthetase.

Although the evidence with regard to a repressor activity of glutamine on the synthesis of glutamine synthetase is more equivocal, in *Ps. fluorescens* organisms, at least, substantial amounts of both glutamine and the enzyme were present intracellularly when these organisms were limited in their growth by the availability of ammonia. Thus any

repressor activity that glutamine might possess must be slight.

From the data contained in Table 1 it must be concluded that, at least with the Gram-negative bacteria listed above, the pool ammonia concentration alone seemingly regulates synthesis of glutamine synthetase. If this is so, then what might be its physiological significance? Tempest, Meers & Brown (1970a) have observed that ammonia-limited cultures of *A. aerogenes* organisms, unlike glucose-limited organisms, contained little glutamate dehydrogenase (EC 1.4.1.4) and synthesized glutamate by a novel pathway that involved first the amidation of glutamate to glutamine and then the reductive transfer of the glutamine amide nitrogen to 2-oxoglutarate to form a second glutamate molecule. This pathway of synthesis of glutamate (and thereby of amino acid biosynthesis) requires, of course, the participation of glutamine synthetase, but is only needed when glutamate synthesis via glutamate dehydrogenase is impeded.

Glutamine synthetases seemingly have much lower K_m values for ammonia than do bacterial glutamate dehydrogenases (see Umbarger, 1969). Further, the concentrations of ammonia present in nitrogen-limited Gram-negative bacteria generally were below those necessary to half-saturate their glutamate dehydrogenases but well above those that would saturate their glutamine synthetases.

Thus, if the functional significance of glutamine synthetase resides in its capacity to assimilate ammonia efficiently, as the first step towards glutamate synthesis under conditions where glutamate dehydrogenase is ineffective, then it is logical to expect that its synthesis would be regulated solely by the intracellular ammonia concentration, which appears to be the case with some Gram-negative bacteria, at least.

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