MODE OF ACTION OF AZASERINE ON *GAFFKYA HOMARI*[1]

S. AARONSON

Haskins Laboratories and the Biology Department, Queens College, New York, New York

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O-Diazoacetyl-L-serine, an antibiotic isolated from a *Streptomyces* species, inhibits tumors (Clarke *et al.*, 1956; Suguira and Stock, 1955) and many microorganisms (Ehrlich *et al.*, 1954*a*, *b*); it also acts as a mutagen (Hemmerly and Demerec, 1955) and a mitotic poison (Biesele, 1958). In several instances the inhibition of tumors and microorganisms by azaserine may be annulled by known metabolites, so permitting inferences as to the site of action of azaserine. The metabolic sites affected by azaserine seem to fall into two main categories (a) nucleic acid biosynthesis: in pigeon liver enzyme systems (Hartman *et al.*, 1955); in ameba (Nakamura, 1956); in strains of *Escherichia coli* (Bennett *et al.*, 1956; Gots and Gollub, 1956); and in chick embryos (Dagg and Karnofsky, 1956); and (b) amino acid biosynthesis: in *E. coli* (Kaplan and Stock, 1954) and in yeast maltase synthesis (Halvorson, 1954).

Gaffkya homari requires either a purine or *p*-aminobenzoic acid (Aaronson and Rodriguez, 1957) for growth. Purines are synthesized endogenously in the presence of *p*-aminobenzoic acid. This permits the study of the effect of azaserine on endogenous purine synthesis as well as exogenous purine utilization in the same microorganism.

EXPERIMENTAL METHODS

G. homari strain ATCC 10400 was used. Experimental cultures were grown in 10-ml micro-Fernbach flasks containing 2 ml of a chemically defined medium (table 1) for 3 to 5 days at 36 to 37 C. Growth was measured as optical density with the Welch Densichron.

Azaserine was sterilized by Seitz filtration and added to the medium aseptically; filtered azaserine gave more consistent results than did autoclaved azaserine. All chemicals except azaserine were obtained from commercial sources.

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Samples of azaserine were obtained through the generosity of Dr. C. C. Stock of the Sloan-Kettering Institute and Mr. Robert Davis, Yale University Medical School.

RESULTS

Azaserine inhibited the growth of *G. homari* with either a purine or *p*-aminobenzoic acid (table 2). Attempts were made to annul the azaserine inhibition with amino acids, vitamins, and nucleic acid derivatives, alone or in combination, as well as with pyridoxamine, pyridoxine, pyridoxal, and pyridoxal phosphate; of these only several amino acids were effective; glutamine and arginine were the most effective amino acids (table 3).

DISCUSSION

The site of azaserine inhibition is different among the several organisms studied; nucleic acid synthesis is especially vulnerable. Purine biosynthesis is readily blocked: azaserine interferes with (a) the incorporation of formate-C^{14} into purines in *E. coli* (Tomisek and Skipper, 1958); (b) the incorporation of glycine-C^{14} into purines but not proteins in ascites tumor cells (Fernandes *et al.*, 1956); and (c) purine biosynthesis in pigeon liver homogenates with the accumulation of acyclic ribotide precursors of purines (Hartman *et al.*, 1956). Others have reported that purines annulled the azaserine inhibition: in ameba (Nakamura, 1956); in chick embryos (Dagg and Karnofsky, 1956); and in sarcoma 180 (Clarke *et al.*, 1956).

The 3 and 9 nitrogens of the purine molecule are supplied by the amide group of glutamine (Reichard, 1955); glutamine annulled the inhibition by azaserine in pigeon liver homogenates as shown by the synthesis of inosinic acid (Hartman *et al.*, 1956), glutamine competed for an enzyme site with azaserine (Levenberg *et al.*, 1957), and glutamine reversed the inhibition by azaserine of mouse tissue cultures (Biesele, 1958). The ability to nullify azaserine inhibition

TABLE 1

Medium for the growth of Gaffkya homari

Na_2-glycerophosphate·$5H_2O$	0.1 g
$MgSO_4 \cdot 7H_2O$	0.05 g
NH_4Cl	0.05 g
L-Glutamic acid	0.5 g
Sucrose	1.0 g
Glycerol	0.5 g
Tris(hydroxymethyl)aminomethane	0.5 g
L-Leucine	5.0 mg
L-Cystine	1.0 mg
DL-Methionine	5.0 mg
Ca pantothenate	0.2 mg
Thiamin·HCl	0.2 mg
Nicotinic acid	0.2 mg
Biotin	1.0 μg
Mo (as $Na_2Mo_7O_4 \cdot 2H_2O$)	0.8 mg
Ca (as chloride)	1.0 mg
Trace metals solution*	1.0 ml
Distilled water to 100 ml; final pH 7.4	

* Formula in Aaronson (1955).

TABLE 2

Effect of varying concentrations of azaserine on the growth of Gaffkya homari in the presence and absence of arginine

Azaserine mg %	Optical Density					
	Control			L-Arginine, 0.2%		
	Hyp.* 1.0 mg %	Ad. 1.0 mg %	PAB 1.0 μg %	Hyp. 1.0 mg %	Ad. 1.0 mg %	PAB 1.0 μg %
0	0.36	0.42	0.36	0.56	0.60	0.56
0.1	0.36	0.36	0.36	0.52	0.56	0.52
0.3	0.28	0.32	0.28	0.44	0.48	0.40
1.0	0.32	0.32	0.32	0.48	0.48	0.48
3.0	0.12	0.12	0.24	0.38	0.40	0.40
6.0	0.04	0.08	0.04	0.08	0.28	0.50
10.0	0	0.04	0.02	0.02	0.08	0.48

* Hyp. = hypoxanthine; Ad. = adenine; and PAB = *p*-aminobenzoic acid.

and the close resemblance of the molecules of azaserine and glutamine (figure 1) have led several workers (Hartman *et al.*, 1956; Biesele, 1958) to believe that azaserine blocks the transfer of nitrogen from glutamine to a purine precursor.

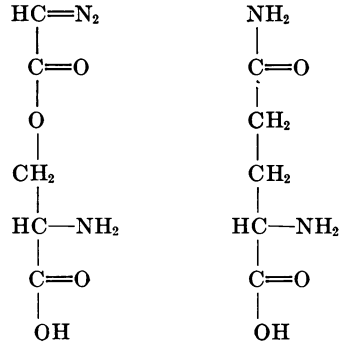
Were this the action of azaserine on *G. homari*, one would expect that cells given exogenous purines would not be inhibited or would be partly inhibited by azaserine whereas cells given *p*-aminobenzoic acid, and presumably forced to synthesize their purines, would be inhibited Azaserine inhibited *G. homari* more with exogenous purines than when purines were synthesized (table 2).

TABLE 3

Comparison of the ability of amino acids to annul azaserine inhibition of Gaffkya homari

Compound*	Molar Conc	Optical Density	
		Control	Azaserine, 10.0 mg %
Hypoxanthine	0.00015	0.40	0
+ L-glutamic acid	0.004	0.45	0
	0.014	0.45	0.21
	0.04	0.54	0.43
+ L-glutamine	0.004	0.53	0.45
	0.018	0.62	0.49
+ L-arginine	0.0002	0.38	0.12
	0.0004	0.42	0.28
	0.004	0.40	0.30
	0.008	0.40	0.32
	0.02	0.49	0.36

* Basal medium contains 0.04 M L-glutamate.



L-Azaserine L-Glutamine

Figure 1. Comparison of the structures of azaserine and glutamine

The only compounds which annulled the azaserine inhibition were L-arginine, L-glutamic acid, and L-glutamine. On a molar basis, arginine was more effective than glutamic acid (table 3) and almost as effective as glutamine. The high activity of arginine may come from its ability to provide 2 moles of glutamine (or glutamate) reported in gram-positive bacteria by Scher and per mole of arginine via an ornithine transaminase Vogel (1957).

Despite the previous evidence for glutamine

TABLE 4

Organisms whose inhibition by azaserine is overcome by amino acids

Organism	Amino Acids		Reference
Kloeckera brevis	Glycine Isoleucine Tyrosine Histidine Lysine Alanine Arginine	Tryptophan Leucine Methionine Phenylalanine Threonine Valine	Reilly (1954*b*)
Escherichia coli strain B	Cystine Phenylalanine Tyrosine Tryptophan		Reilly (1954*b*)
E. coli (Waksman strain)	Tryptophan Phenylalanine Tyrosine		Kaplan and Stock (1954)
E. coli	Phenylalanine Tryptophan		Tomisek and Skipper (1958)
E. coli mutant	Tryptophan Phenylalanine Tyrosine	Glutamic acid Glutamine Leucine	Gots and Gollub (1956)
Yeast (maltase synthesis)	Leucine Isoleucine Norleucine Phenylalanine	Tyrosine Methionine Valine	Halvorson (1954)

nitrogen transfer as the site of azaserine inhibition, there may be others. Some workers reported that glutamine did not nullify azaserine inhibition: in ascites tumor cells (Fernandes *et al.*, 1956); in sarcoma 180 (Clarke *et al.*, 1956); and in chick embryos (Dagg and Karnofsky, 1956). Besides glutamine and the amino acids reported here, many other amino acids suppress azaserine inhibition in various biological systems (table 4).

It appears now that the main action of azaserine is interference with nitrogen transfer in purine and amino acid synthesis, perhaps by blocking transaminase reactions in which most amino acids participate (Meister, 1955). Barker *et al.* (1956) reported a marked increase in glutamine and acids of the tricarboxylic acid cycle in Scenedesmus but not Chlorella inhibited by azaserine; they attributed this to interference with transamination. Reilly (1954*a*) found azaserine inactivated by mouse liver preparations. The enzyme seemed to be a deaminase since azaserine lost its ninhydrin reaction, ultraviolet absorption, and inhibitory ability. It appears that the amino group of azaserine as well as its steric configuration are necessary for its ability to inhibit.

Since the B_6 vitamins play an important role in nitrogen metabolism an attempt was made to determine whether the coenzyme form, pyridoxal phosphate, as well as several of its more stable analogues affected azaserine inhibition; they did not.

SUMMARY

O-Diazoacetyl-L-serine (azaserine) inhibited the multiplication of *Gaffkya homari* equally well in the presence of exogenous purines as when purines were synthesized endogenously (in *p*-aminobenzoic acid-containing media). Purine synthesis is not the main site of inhibition by azaserine in this microorganism. Glutamine,

arginine, and glutamic acid annulled the inhibition by azaserine. The role of azaserine in nitrogen transfer is discussed.

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