ARGININE INHIBITION OF GLUTAMATE UTILIZATION

by

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Arginine specifically inhibits the utilization of lysine by a lysineless mutant of *Neurospora crassa* (No. 4545), the index for half-maximal inhibition being approximately one[1].

In the present investigation, which is a part of a systematic study of amino acid antagonisms, arginine has been found to inhibit competitively the utilization of glutamate and less effectively glutamine in *Streptococcus lactis* 8039. The effect of proline on the inhibition is consistent with the interpretation that arginine prevents the utilization of either glutamate or glutamine in the biosynthesis of proline. A study of this inhibition and its relationship to aspartic acid as an inhibitor of glutamate utilization was undertaken.

EXPERIMENTAL METHODS

The assay procedure and medium** were the same as previously described[2] except that the medium was modified to contain no arginine, 5 mγ of biotin per 5 ml, and 20 γ each of adenine, guanine and uracil per 5 ml. Glutamine, when supplemented as indicated in the Tables, was dissolved in sterile water and added aseptically to the autoclaved medium. Cultures of *Streptococcus lactis* 8039 grown in 10 ml of yeast extract-peptone medium for 20 to 24 hours at 30° were used to inoculate assays as previously described[2].

RESULTS

The minimum toxic concentration of arginine is 0.5 mg per 5 ml for *S. lactis* 8039 in the basal medium containing 0.2 mg of DL-glutamic acid per 5 ml, and as little as 1 γ of glutamine per 5 ml prevents the inhibition of this level of arginine. Glutamine reverses the inhibition in a competitive manner, as indicated in Table I, such that the ratio of arginine to glutamine necessary for inhibition of growth is approximately 2000.

As shown in Table II, glutamic acid also prevents the toxicity of arginine in a manner similar to glutamine but is much less effective. The ratio of arginine to glutamic acid at which inhibition of growth occurs is slightly variable from 2.5 at the lower levels of glutamic acid to 5 at the higher levels. Increasing the concentration of glutamic acid from 0.2 to 0.5 mg per 5 ml has only a slight effect on the amount

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** Calcium pantothenate (3 mg per 30 ml of vitamin supplement) was inadvertently omitted from the list of constituents of the basal medium previously described[2].

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TABLE I

REVERSAL OF ARGININE INHIBITION BY GLUTAMINE

Test organism, *Streptococcus lactis* 8039, incubated 24 hours at 30°.

L-*Arginine* mg per 5 ml	*Supplements* L-*Glutamine*, γ per 5 ml					
	0	1	2	5	10	20
	Galvanometer readings*					
0.1	40					
0.2	38	42				
0.5	9	41	41			
1.0	3	37	41	43		
2		4	26	43	44	
5			1	17	42	48
10				5	22	44
20					10	29
50						6

* A measure of culture turbidity; distilled water reads 0, an opaque object 100.

TABLE II

EFFECT OF GLUTAMIC ACID ON ARGININE TOXICITY

Test organism, *Streptococcus lactis* 8039, incubated 24 hours at 30°.

L-*Arginine* mg per 5 ml	*Supplements* DL-*Glutamic acid**, mg per 5 ml							
	0.2	0.5	1.0	2	5	0.2	0.5	0.2
	L-*Glutamine*, γ per 5 ml							
	0	0	0	0	0	20	20	50
	Galvanometer readings							
0.1	40							
0.2	38							
0.5	9	55						
1.0	3	48	55					
2		6	55	58				
5			6	44	55	47	50	55
10				9	57	33	50	54
20					15	21	27	44
50					3	6	5	21

* Glutamic acid omitted from basal medium.

of arginine necessary for inhibition of growth of the organism in the presence of 20 γ of glutamine per 5 ml. However, increasing the concentration of glutamine from 20 γ to 50 γ per 5 ml increases approximately two-fold the amount of arginine necessary for inhibition of growth in the presence of 200 γ of glutamic acid per 5 ml. Separate experiments indicate no more than an additive effect of combinations of glutamine and glutamic acid in reversing the arginine toxicity.

The effect of proline on arginine inhibition of the utilization of glutamine is indicated in Table III. If proline is omitted from the basal medium, the inhibition index determined over a ten-fold range of glutamine concentration is approximately 1000. In the presence of a supplement of proline, the inhibition index is increased slightly more than two-fold over that determined in the absence of exogenous proline.

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TABLE III

THE EFFECT OF PROLINE ON ARGININE INHIBITION

Test organism, *Streptococcus lactis* 8039, incubated 24 hours at 30°.

L-*Arginine* *mg per 5 ml*	L-*Glutamine* *γ per 5 ml*	*Supplements** *None*	L-*Proline* *100 γ per 5 ml*	L-*Arginine* *mg per 5 ml*	L-*Glutamine* *γ per 5 ml*	*Supplements** *None*	L-*Proline* *100 γ per 5 ml*
		Galvanometer readings				Galvanometer readings	
0.2	2	40		1	10	42.5	
0.5	2	37.5	44	2	10	39.5	49
1	2	17.5	43	5	10	13	47
2	2	5	29	10	10	8	20
5	2		8	20	10		12
0.5	5	43		2	20	44.5	50
1	5	40	49	5	20	38	53
2	5	27.5	47.5	10	20	18	45
5	5	6.5	25	20	20	13	25
10	5		9				

* Proline omitted from basal medium.

As indicated in Table IV, aspartic acid exerts inhibitory effects on *S. lactis* 8039 similar to the toxicities observed with *Lactobacillus arabinosus* 17-5[3]. Glutamic acid prevents the toxicity of aspartic acid in a similar manner for both organisms in that the reversal is not strictly competitive, a two-fold increase in aspartic acid concentration for each 50% increase in glutamic acid concentration being necessary for inhibition of growth of the organisms.

TABLE IV

EFFECT OF GLUTAMIC ACID ON ASPARTIC ACID TOXICITY

Test organism, *Streptococcus lactis* 8039, incubated 21 hours at 30°.

DL-*Aspartic acid** *γ per 5 ml*	DL-*Glutamic acid** *γ, per 5 ml* 250	400	500	750	1000	1500
	Galvanometer readings					
100	42	48	49	50	50	50
2000	34	37				
5000	13	22				
10000		18	30	41	45	48
20000		13	25	33	43	46
50000			14	25	36	44

* Glutamic acid, proline, and aspartic acid omitted from basal medium supplemented with 50 γ L-arginine per 5 ml.

Also, in separate experiments with *S. lactis* 8039 proline increases the ratio of aspartic acid to glutamic acid necessary for inhibition of growth analogous to the effect observed with *L. arabinosus* 17-5[3].

The effect of glutamine on aspartic acid toxicity for *S. lactis* 8039 paralleled that obtained with *L. arabinosus*. Since glutamine is (500 times) more effective than

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glutamic acid in preventing the toxicity of aspartic acid, it appeared likely that aspartic acid and arginine inhibit the same or closely related functions. To determine whether the same or different mechanisms of action of the two inhibitors were involved, the effect of arginine on aspartic acid toxicity was investigated. Results shown in Table V indicate a synergistic action for the two inhibitors. A combination of as little as one-fifth the amount of aspartic acid and one-tenth the amount of arginine separately necessary for inhibition of growth prevents the growth of *S. lactis* 8039. Similar experiments in which proline was omitted from the basal medium also showed synergistic inhibitory effects of combinations of arginine and aspartic acid.

TABLE V

SYNERGISTIC EFFECT OF ARGININE ON ASPARTIC ACID TOXICITY*

Test organism, *Streptococcus lactis* 8039, incubated 24 hours at 30°.

DL-*Aspartic acid* *mg per 5 ml*	L-*Arginine, γ per 5 ml*				
	50	100	200	500	1000
		Galvanometer readings			
0.1	53	55	51	34	6
1.0		50	49	20	
2		44	33	10	
5		33	10	3	
10		9	8		
20	37	8			
50	11				
100	5				

* DL-Glutamic acid increases to 250 γ per ml.

DISCUSSION

Arginine prevents the utilization of either glutamic acid or glutamine in a competitive manner; however, glutamine is from 500 to 1000 times as effective as glutamic acid in preventing the inhibition. Since glutamine and glutamic acid are each competitive reversing agents for the same inhibitor and combinations of glutamine and glutamic acid exert additive rather than synergistic effects, these metabolites appear to compete (or form substances which compete) with arginine for a specific site. Glutamine is more easily utilized to interact with the specific site than is glutamic acid under the testing conditions.

Since supplements of proline at moderate concentrations increase the ratio of arginine to glutamine necessary for inhibition of growth, proline probably either allows growth by exerting a sparing effect on the product of the inhibited system or is the product of the first inhibited reaction. In either case, the results are consistent with the interpretation that arginine prevents the utilization of glutamate for the biosynthesis of proline.

The synergistic effect of aspartic acid and arginine indicate that different mechanisms of inhibition of utilization of glutamate are involved, presumably involving separate sites functioning in sequence[4]. Results reported elsewhere show that glutamine at low concentrations increases the aspartate-glutamate ratio necessary for growth inhibition and at higher concentrations exerts an additional additive

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effect with glutamate[3]. Since no such synergistic effect is noted with glutamine and glutamic acid in reversing arginine toxicity, it appears that arginine exerts its effect at a stage such that glutamine can only compete with the inhibitor, while aspartic acid inhibits at a stage such that glutamine can exert a sparing effect on the inhibited enzyme system. These results are consistent with the interpretation that aspartic acid exerts its effect prior in the sequence to arginine.

SUMMARY

Arginine competitively inhibits the utilization of either glutamic acid or glutamine in *Streptococcus lactis* 8039, the inhibition indices for maximal inhibition being 2–5 and approximately 2000, respectively. Combinations of glutamic acid and glutamine exert additive rather than synergistic effects in reversing the inhibition. Proline increases the inhibition index approximately two-fold over a 10-fold range of concentrations of glutamine. Aspartic acid, the toxicity of which is also reversed by glutamic acid or glutamine, exerts a synergistic effect with arginine in inhibiting *S. lactis* 8039. These results are consistent with the interpretation that arginine competitively prevents the utilization of either glutamate and less effectively glutamine for biosyntheses including that of proline by a mechanism different from aspartic acid inhibition.

RÉSUMÉ

L'arginine inhibe compétitivement l'utilisation soit de l'acide glutamique soit de la glutamine par *Streptococcus lactis* 8039, les indices d'inhibition pour l'inhibition maximum étant respectivement de 2–5 et d'environ 2000. Dans des mélanges, l'acide glutamique et la glutamine exercent des effets additifs plutôt que synergiques sur le renversement de l'inhibition. La proline augmente l'indice d'inhibition d'environ deux fois, les concentrations en glutamine variant entre elles de 1 à 10. L'acide aspartique, dont la toxicité est également supprimée par l'acide glutamique ou la glutamine, exerce un effet synergique de celui de l'arginine sur l'inhibition de *S. lactis* 8039. Ces résultats sont en accord avec l'hypothèse selon laquelle l'arginine empêcherait compétitivement l'utilisation du glutamate et, à un degré moindre, de la glutamine, dans des biosynthèses, entre autres celle de la proline, par un mécanisme différent de celui de l'inhibition par l'acide aspartique.

ZUSAMMENFASSUNG

Arginin wirkt kompetitiv hemmend auf den Glutaminsäure- oder Glutaminverbrauch von *Streptococcus lactis* 8039; die Hemmungsindexe für die maximale Hemmung betragen 2–5, bezw. ungefähr 2000. Die vereinigte Wirkung von Glutaminsäure und Glutamin auf die Aufhebung der Hemmung ist eher additiv als synergistisch. Prolin erhöht den Hemmungsindex, über ein zehnfaches Konzentrationsgebiet von Glutamin, ungefähr auf das Zweifache. Asparaginsäure, deren Toxizität gleichfalls von Glutaminsäure oder Glutamin rückgängig gemacht wird, übt mit Arginin eine synergistische Wirkung auf die Hemmung von *S. lactis* 8039 aus. Diese Ergebnisse stimmen mit der Hypothese überein, dass Arginin den biosynthetischen Verbrauch von Glutamat oder, in geringerem Masse, von Glutamin, sowie von Prolin kompetitiv verhindert, und zwar durch einen Mechanismus, welcher sich von der Asparaginsäurehemmung unterscheidet.

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