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THE ROLE OF SPECIFIC AMINO ACIDS IN THE PROTECTION OF *E. COLI* AGAINST B—LACTAM ANTIBIOTICS *IN VITRO*

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ABSTRACT

The role of specific amino acids namely cysteine, methionine, threonine and asparagine in the protection provided by vamin solution against B-lactam inhibition to *E. coli* was evaluated *in vitro* In minimal medium, cells were treated with 32 ug/ml of penicillin G, carbencillin, hostacillin, cloxacillin and cephalotin in the presence of specific amino acid supplementation. Deletion of specific amino acids from the media abolished the protection provided by vamin. Threonine was essential for the protection of cells against all tested antibiotics, while cysteine was essential for protection against carbencillin and sephalotin. Deletion of methionine or asparagine abolished the protection against carbencillin and to a less extent cephalotin.

INTRODUCTION

Many patients who receive treatments with the B-lactam antibiotics are subject to the administration of nutritional supplementation such as vamin solution. This solution contains 18 amino acids as well as glucose and electrolites. Possible interference of this supplementation with the activity of several B-lactam antibiotics was previously evaluated in *E. coli* in vitro (Ammash and Kassim, 1989). Significant inactivation of up to 64 ug/ml of carbencillin, cephalotin, penicillin G, and hostacillin was induced by addition of vamin to the culture media. Group deletion of specific amino acids revield that the branched chain amino acids (valine, leucine and isoleucine) were essential for the inactivation of carbencilin and cephalotin. Aromatic amino acids (tyrosine, tryptophan and phenylalanine) were essential for the inactivation

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of cephalotin only. Amino acids were also found to protect bacterial cells from the toxicity of other factors such as hyperbaric oxygen (Boihme *et all.* 1976, and aerobic paraqual (Heitkamp and Brown. 1982). The study was undertaken to determine the role of other amino acids specifically cysteine, methionine, threonine and asparagine in the protection that was provided by vamin to *E. col.* against the toxicity of carbencillin, penicillin G, hostacillin, cloxacillin and cephalotin.

MATERIALS AND METHODS

Culture :

Local isolate of *Escherichia coli* was grown at 37 C in minimal basai salts (MBS)-glucose medium (Brunker and Brown, 1971), or in this medium supplemented with vamin solution (Kabi Vitrum, Stockholm, Sweden), or with specific amino acids at 0.65 mM each.The medium was sterilized by using millipore HAWP, 0.45 um pore size membranes. Cellular growth was monitored by measuring changes in absorbance at 500 nm.

Amino Acid Supplementation :

Vamin solution was added as 1 : 20 v/v to sterile MBS-glucose medium. This solution contains valine, leucine, isoleucine, tyrosine, tryptophan, phenylalanine, alanine, cysteine, methionine,threonine. glycine, proline, lycine histidine, arginine. glutamine and glutamic acid at concentrations ranging from 2.4 mM to 60 mM. Amino acids (BDH) were supplemented to the media as described by Ammash and Kassim, (1989).

Antibiotic Exposure :

Fifty ml of bacterial culture at mid-exponential phase were treated with 32 u/ml of carbencillin, cloxacillin (Beecham Res. Int., Middlesex, England), hostacillin. penicillin G (Hoechest AG, Frankfurt am Main. Germany), or cephalotin (Eli Lilly and Co., Indianapolis, Ind., U.S.A.). The growth was monitored by measuring changes in absorbance at 500 nm every 30 min. Sensitivity tests were performed according to Bauer, *et. al.* (1966) prior to and following each experiment to exclude mutants.

RESULTS

The effect of the deletion of 4 amino acids on the generation time of antibiotics treated *E. coli* is shown in table (1). Deletion of cysteine caused

Table (1)

Contribution of Individual Amino Acid to Protection of *E. coli* from β-lactam Antibiotics.

Deleted amino acid	Antibiotic treatment[a]					
	None	Penicillin G	Carbencillin	Hostacillin	Cloxacillin	Cephalotin
	Average of generation time/hr. ± S.D.[b]					
None[c]	0.70 ± 0.09	0.89 ± 0.22	2.11 ± 1.25	1.35 ± 0.74	1.48 ± 1.12	2.30 ± 0.01
Cysteine	0.98 ± 0.45	0.98 ± 0.74	NG[d]	1.25 ± 0.49	1.62 ± 0.44	NG
Methionine	0.92 ± 0.45	1.12 ± 0.33	Lysis	1.49 ± 0.44	2.49 ± 1.11	0.96 ± 0.52*
Threonine	0.97 ± 0.52	NG	Lysis	NG	NG	NG
Asparagine	0.96 ± 0.21	2.50 ± 2.31	NG	1.99 ± 1.11	0.97 ± 0.36	1.95 ± 0.6

a) Bacterial cells were grown in MBS-glucose medium at 37 Cin a shaker incubator at 120 rpm. Cells were treated with antibiotic at 32 ug/ml mid-exponential phase. Amino acidswere added at 0.65 mM each

b) Average generation time/hr. after 2 hr monitoring period following treatment with antibiotics.

c) Vamine was added to the medium.

d) Denotes n_O growth over sampling intervals.

complete ihibition of carbencillin (figure 1) and cephalotin (figure 2) treated cells. The deletion of methiomine caused lysis of carbencillin treated cells, and induced growth inhibition after 2 hours of treatment with cephalotin. Less effect was observed with cloxacillin as only growth delay was induced which was indicated by 168% increase in the vamin solution to hostacillin or penicillin G treatment was not altered by the absence of methionine from the media (figure 1). Deletion of threonine on the other hand caused inhibiton to the growth of all cultures. The strongest effect was observed with carbencilin treatment as indicated by the lysis of the cells (figure 1). The absence of asparagine also caused lysis of cells treated with carbencillin and with cephalotin but only after 2 hours of treatment with the later (figure 1, 2). Deletion of this amino acid induced a growth delay to the culture treated with penicillin G as generation time increased 284%.

DISCUSSION

Previous findings have revealed that vamin nutritional solution interferes with the activity of B - lactam antibiotic against *E. coli* in vitro (Ammash and Kassim, 1989). This group of antibiotics inhibit cellular growth by preventing the maturation of peptidoglycan layer as a result of the inhibition of the cross linking between amino acid chains in the cell wall (Gottlieb and Shaw, 1967; Lancini and Parenti, 1982). The protection that was provided by vamin to *E. coli* was due to the pressence of specific amino acids in the solution. Among them is valine, leucine, isoleucine, tyrosine, tryptophan, and phenylalanine.. In this study it was found that the deletion of cysteine or methionine from the media of antibiotic treated *E. coli* resulted in a total loss of the protection provided by vamin from carbencillin and cephalotin. The deletion of asparagine caused loss of the protection from the inhibitory effect of carbencillin and to less extent penicillin G and cephalotin. Threonine on the other hand showed the strongest effect. Its deletion from the medium resulted in growth inhibition of all antibiotic treated cultures. These findings are in agreement with previous results found in *E.coli* exposed to gentamicin or streptomycin (Ammash and Kassim, under publication), and to hyperoxia (Biohme, *et al.*, 1976), and to paraquat (Heitkap and Brown, 1982). The protection provided by amino acids solution from the toxicity of hyperoxia and paraquat was found to be due to their circumvention to the inhlbitory efffect of these agents to the biosynthes's of amino acids.

This results from the inactivation of specific enzymes in the pthway such as dihydroxyacid dehydratese in the cosynthetic pathaw of the branched chain amino acids (Brown and Yein, 1978; Fee *et al.*, 1980). According to the data presented in this study it is proposed that the protection provided by vamin from the inhibitory effect of specitic B-lactam (carbencillin, cephalotin penicillin G, hostacillin, and cloxacillin) to *E. coli* is probably due to the presence of specific amino acids in the solution. Among them is threonine, cysteine, and to less extent methionine and asparagine. The presence of these amino acids circumvent the inhibitory effect of these antibiotics probably by acting as analogues to them and therefore they bind with the enzymes D-alanine carboxypeptidase and peptidoglycan transpeptidase and prevent the formation of the antibiotic-enzyme complex; (which ususlly inhibit the enzymes activity) and therefore they allow the continuation of cell wall synthesis and cellular growth. The circumvention of the inhibitory effect of the B-lactam antibiotics to *E. coli* that was provided by amino acids suggests that these antibiotics might induce inhibition to the biosynthetic pathway of these amino acids by inactivating specific enzymes in a manner similar to that previously found in hyperoxia and paracuat treated *E. coli*.

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دور احماض امينية خاصة في حماية *E. coli* من مضادات البيتالاكتام في الزجاج

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الخلاصة

لقد تمت دراسة دور الاحماض الامينية وخاصة السيستايين والميثايونين والثريونين والاسباراجين في الحماية التي يوفرها محلول الفامين المغذى ضد تأثير مضادات البيتالاكتام المثبط لنمو *E. coli* في الزجاج · لقد عوملت الخلايا المنماة في وسط الحد الادنى بـ ٣٢ ميكروغرام/مل من بنسلين ج والكاربنسلين والهوستاسلين والكلوكساسلين والسيفالوتين وذلك بتوفر الاحماض الامينية · ادى حذف احماض امينية خاصة في الوسط الزرعي الى الغاء هذه الحماية · لقد كان الثريونين اساسي لحماية الخلايا من كل المضادات المختبرة · بينما كان السيستايين اساسي للحماية من الكاربنسلين والسيفالوتين · ادى حذف الميثايونين والاسباراجين الى الغاء الحماية من الكاربنسلين وبدرجة أقل السيفالوتين ·

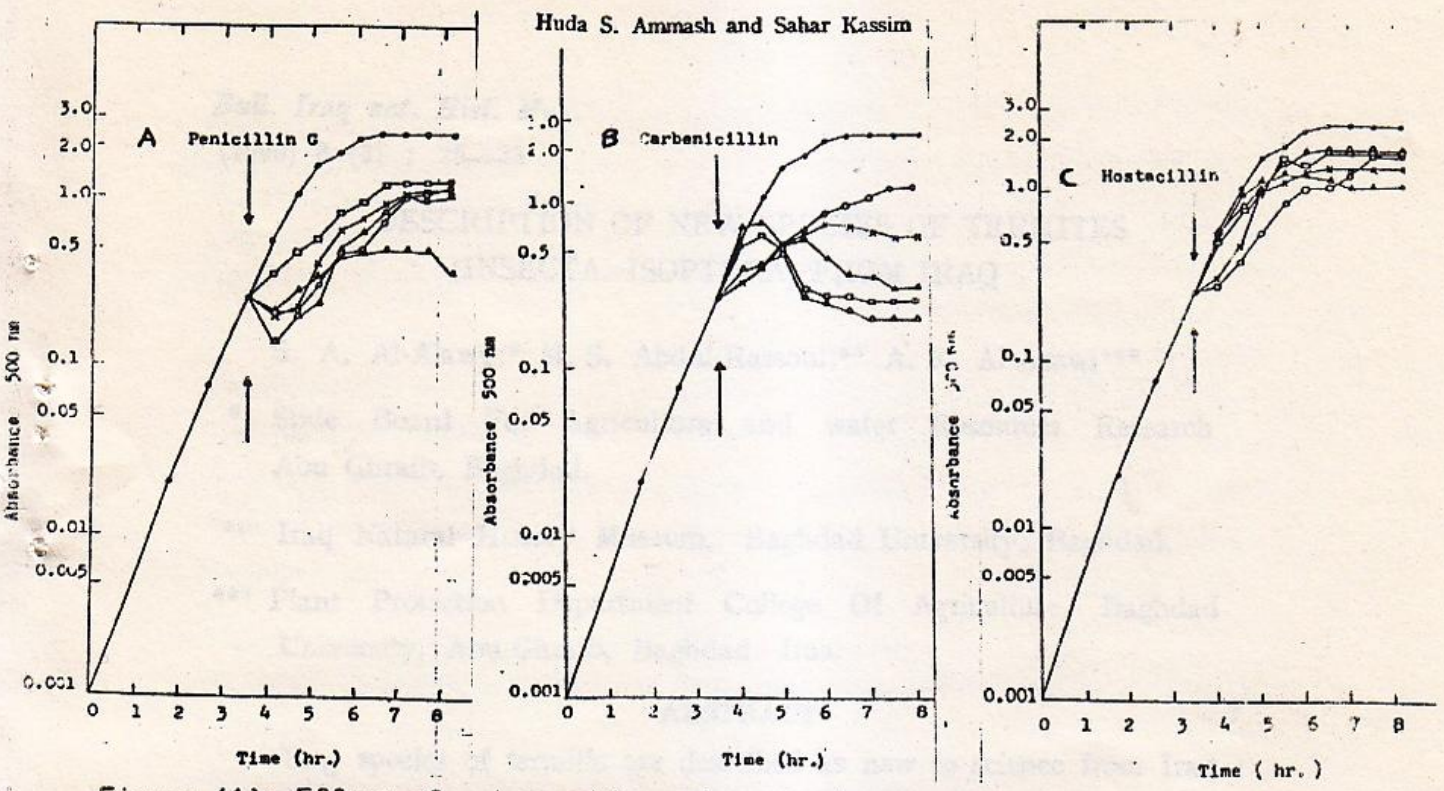

Figure (1): Effect of amino acid supplementations on the growth of penicillin G, carbencillin, and hostacillin treated E. coli. The deleted amino acids are; ● none, o all. □ cysteine, △ methionine, ▲ threonine, × asparagine.

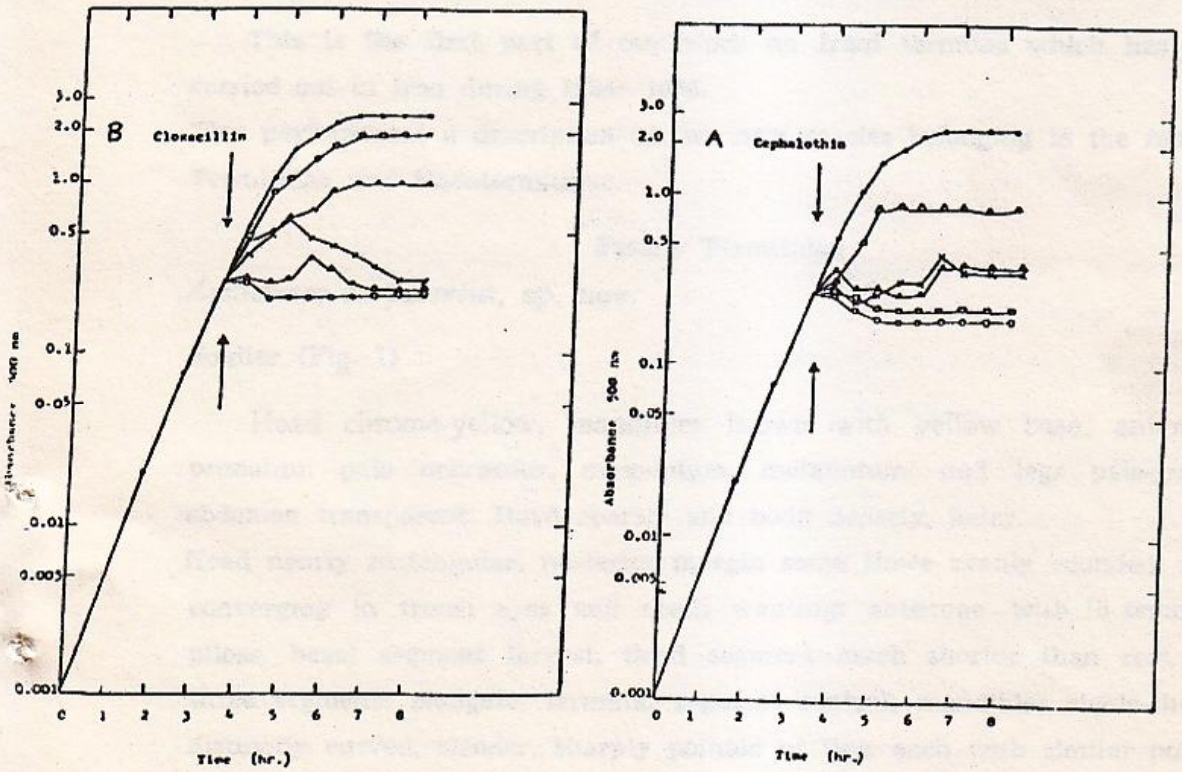

Figure 2: Effect of amino acid supplementations on the growth of cephalothin and cloxacillin treated E. coli. Deleted amino acids are; ● none, o all, □ cysteine, △ methionine, ▲ threonine, × asparagine.