THE LYSIS OF *BACTERIUM COLI* BY AMINO-ACIDS

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(With 2 Figures in the Text)

Gordon, Hall & Stickland (1951, 1953) described the kinetics of the lysis by glycine of *Bacterium coli* suspensions, and the inhibition by various agents of this process. The present paper attempts to correlate this lytic effect with the chemical structure of various amino-acids.

MATERIALS AND METHODS

Preparation of bacteria

Four freshly isolated strains of *Bact. coli* were grown on the surface of nutrient agar for 16–18 hr. at 37°, and were then washed off with distilled water. These suspensions were filtered through glass wool. The bacteria were washed twice and finally suspended in about ten times their volume of distilled water which was equivalent to a concentration of between 10 and 20 mg. dry weight of cells per ml.

Measurement of lysis

After the suspensions had been incubated with the amino-acid solutions under test the bacteria were removed by centrifuging at 2500 g. for 20 min. The supernatant fluid was treated with $\frac{1}{4}$ volume of 25 % 'w/v' trichloracetic acid solution, the protein precipitate was washed with 5 % trichloracetic acid and then the protein was estimated by the biuret method (Robinson & Hogden, 1940). The degree of lysis was expressed as the ratio of the protein liberated into the supernatant fluid to the total soluble protein of the bacterial suspension (Stickland, 1951).

Amino-acids used

Glycine, DL-alanine, β-alanine, DL-α-amino-*n*-butyric acid, DL-α-amino-*iso*-butyric acid, DL-α-amino-*n*-valeric acid, DL-valine, DL-serine, glycine ethyl ester hydrochloride, aceturic acid, glycylglycine and L-glutamic acid were obtained commercially. γ-Amino-*n*-butyric acid was prepared by the method of De Witt (1943), DL-$\alpha\beta$-diamino-*n*-butyric acid by the method of Neuberg (1906), DL-$\alpha\gamma$-diamino-*n*-butyric acid by the method of Adamson (1939), and DL-$\alpha\beta$-diaminopropionic acid by the method of Bergmann & Grafe (1930). DL-alanine was resolved by the method of Pacsu & Mullen (1940); the products showed the following rotations at a concentration of 4 % in N HCl: for the L-isomer $\alpha_{20}^{D} = +14{\cdot}5°$; for the D-isomer, $\alpha_{20}^{D} = -14{\cdot}4°$.

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RESULTS

The effect of blocking the amino- and carboxyl-groups of glycine

The lytic activities of acetylglycine (aceturic acid), glycine ethyl ester, and glycylglycine were compared with that of glycine at the same molar concentration. Table 1 shows that any interference with the integrity of the amino- or carboxyl-group, or their separation by the interposition of a peptide bond, caused loss of lytic activity.

The effect of the amino-acid chain length

The lytic activities of the straight chain amino-acids glycine, DL-alanine, DL-α-amino-*n*-butyric acid, and DL-α-amino-*n*-valeric acid were compared. Table 2 shows that if these substances were at equal molar concentrations, they caused an equal amount of lysis, and Fig. 1 shows that the lysis followed a similar course whichever of these amino-acids was used.

Table 1. *Failure of various derivatives of glycine to lyse* Bact. coli

	Lysis %	
Amino-acid derivative	Exp. 1	Exp. 2
Glycine	52	63
Glycine ethyl ester	1	7
Acetyl glycine	0	6
Glycylglycine	3	—

Lysis was measured after incubation of the cell suspension for 16 hr. at 37° C. in *M* solution of the amino-acid derivative at pH 7·5. Each experiment gives the average lysis of four strains.

Table 2. *Effect of length of chain amino-acids on the lysis of* Bact. coli

	Lysis %		
Amino-acid	Strain A	Strain B	Strain D
Glycine	—	51	64
DL-alanine	65	53	75
DL-α-amino-*n*-butyric acid	72	51	73
DL-α-amino-*n*-valeric acid	67	—	67

Lysis was measured after incubation of the cell suspension for 16 hr. at 37° in *M* solution of the amino-acid at pH 7·5, except for DL-α-amino-*n*-valeric acid which was used at 0·5 *M*.

The effect of the optical isomers of alanine

DL-, L-, and D-alanine were compared for their lytic activity. Table 3 shows that L- and D- isomers were equally potent in causing lysis.

The effect of the position of the amino-group

Two pairs of amino-acids α- and β-alanine and α- and γ-amino-*n*-butyric acid were tested for lytic activity. In each pair the lytic activity was greatly reduced when the amino-group was not in α-position (Table 4). The presence of a second amino-group in addition to that in the α-position in αβ-diamino-propionic acid and αβ- and αγ-diamino-butyric acid, still further reduced the activity, as did the

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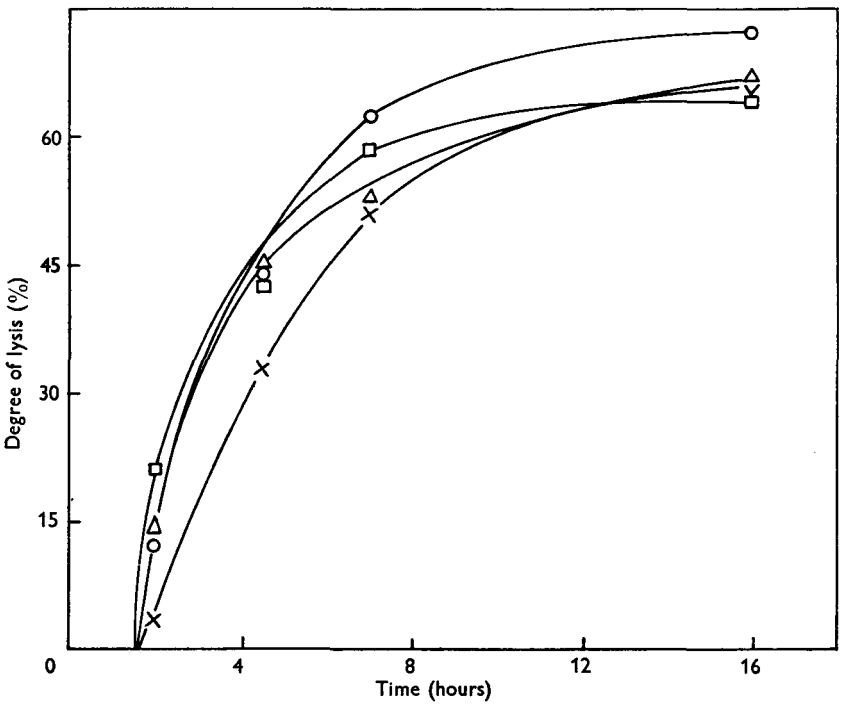

Fig. 1. The course of the lysis of *Bact. coli* by *n*-α-amino-acids. □——□, glycine; ×——×, DL-alanine; ○——○, DL-α-amino-*n*-butyric acid; △——△, DL-α-amino-*n*-valeric acid.

Table 3. *Lytic activity of optical isomers of alanine*

Amino-acid	Lysis % Strain B	Strain C	Strain D
DL-alanine	66	66	69
L-alanine (natural)	65	67	65
L-alanine (resolved)	53	59	59
D-alanine (resolved)	64	63	64

Lysis was measured after incubation of the cell suspension for 16 hr. at 37° in *M* solution of the amino-acid.

Table 4. *Effect of varying the position of the amino-group on the lytic activity of amino-acids*

Amino-acid *Bact. coli* strain ...	Lysis % A	B	C	D	A	B
DL-alanine	58	50	51	51	74	53
β-alanine	15	14	12	12	—	27
DL-αβ-diaminopropionic acid	0	0	0	0	1	—
DL-α-amino-*n*-butyric acid	58	56	52	53	68	51
γ-amino-*n*-butyric acid	13	10	10	8	—	29
DL-αβ-diamino-*n*-butyric acid	1	1	0	2	9	—
DL-αγ-diamino-*n*-butyric acid	2	2	1	1	1	—
L-glutamic acid	1	0	0	0	11	—
DL-serine	2	5	6	2	5	—

Lysis was measured after incubation of the cell suspension for 16 hr. at 37° in a *M* solution of the amino-acid at pH 7·5.

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presence of a second carboxyl group in L-glutamic acid (Table 4). The presence of a β-hydroxy group in DL-serine also destroyed the activity (Table 4).

The effect of branching of the carbon chain

Two pairs of amino-acids, DL-α-amino-*n*- and *iso*-butyric acids, and DL-α-amino-*n*- and -*iso*-valeric acids, were compared for their lytic activity. We have previously reported (Gordon, Hall & Stickland, 1952) that DL-α-amino-*iso*-butyric had no

Table 5. *Effect of branching of the chain on the lytic activity of amino-acids*

	Lysis %			
Bact. coli strain ...	D	A	B	D
DL-α-amino-*n*-butyric acid	57	62	51	53
DL-α-amino-*iso*-butyric acid	39	26	28	16
DL-α-amino-*n*-valeric acid	57	53	—	—
DL-α-amino-*iso*-valeric acid	27	27	—	—

Lysis was measured after 16 hr. incubation of the cell suspension at 37° C. in 0·5 *M* solution of the amino-acid at pH 7·5.

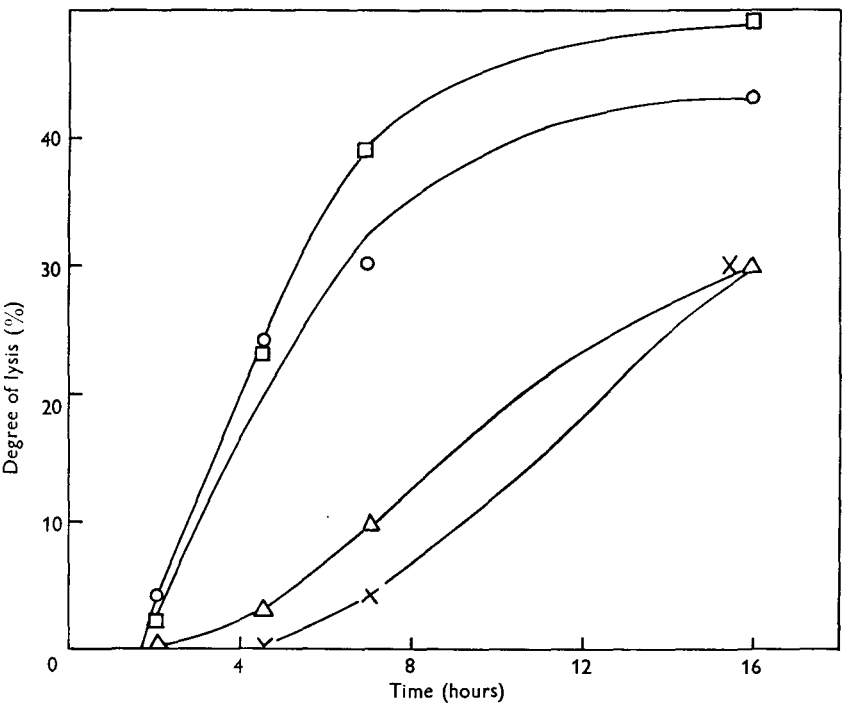

Fig. 2. The course of the lysis of *Bact. coli* by straight- and branched-chain α-amino-acids. □——□, DL-α-amino-*n*-butyric acid; △——△, DL-α-amino-*iso*-butyric acid; ○——○, DL-α-amino-*n*-valeric acid; ×——×, DL-α-amino-*iso*-valeric acid.

lytic activity against some strains of *Bact. coli*. With the strains used in the present study this was not so, and both the branched-chain amino-acids had considerable activity, though less than the straight-chain amino-acids (Table 5).

The difference between the *n*- and *iso*-acids is demonstrated better when the course of the lysis is compared (Fig. 2). The lysis by the branched-chain acids shows a longer lag, and at 5 hr. is much less than that with the straight-chain acids.

Correlation of lytic activity and bactericidal power

Some of these amino-acids were tested for their bactericidal power, since previous tests had shown a correlation between lytic and bactericidal action (Gordon *et al.* 1952). The bactericidal power was measured by inoculating a loopful of a suspension of *Bact. coli* into a series of tubes containing graded concentrations of the amino-acid in nutrient broth, and incubating the tubes at 37° C. for 18 hr. The cultures were then subcultured on to heated blood agar and incubated again. The results (Table 6) confirmed that there existed a fairly close correlation between these two properties.

Table 6. *The bactericidal power of some amino-acids against* Bact. coli

	Concentration of amino-acid (*M*)						
Amino-acid	0·1	0·2	0·3	0·4	0·6	0·8	1·0
Glycine	+ +	+ +	0	0	0	0	0
DL-α-alanine	+ +	+ +	0	0	0	—	—
DL-α-amino-*n*-butyric acid	+ +	0	0	0	0	—	—
Acetyl glycine	—	+ +	—	+ +	+ +	+ +	+ +
Glycylglycine	—	+ +	—	+ +	+ +	+ +	+ +
β-alanine	—	+ +	—	+ +	+ +	—	—
γ-amino-*n*-butyric acid	—	+ +	—	+ +	+ +	+ +	—
DL-αβ-diamino-*n*-butyric acid	—	+ +	—	+ +	+	0	0
L-glutamic acid	—	+ +	—	+ +	+ +	—	—
DL-serine	—	+ +	—	+ +	+ +	—	—
DL-α-amino-*iso*-butyric acid	+ +	+ +	+ +	+ +	+	—	—
DL-α-amino-*iso*-valeric acid	+ +	+	0	0	0	—	—

+ + = confluent growth; + = marked growth; 0 = no growth; − = not tested.

DISCUSSION

The facts that emerge from this study are that the straight-chain α-amino-acids, whether of D- or L- configuration, are equally effective in causing lysis of *Bact. coli*, and that any departure from this structure decreases the activity. Thus branching of the chain reduces the activity slightly, removal of the amino-group from the α- to the β- or γ-position reduces the activity markedly, while blocking the amino-group by acetylation or the carboxy-group by esterification destroys the activity completely. The introduction of other substituents such as amino-, carboxy-, or hydroxy-groups also destroys the activity.

As the optical isomers of the α-amino-acids are equally effective in causing lysis, their role does not appear to be related to their occurrence as natural components of proteins.

SUMMARY

1. Straight-chain α-amino-acids are all equally effective in causing the lysis of *Bact. coli*.

2. Various modifications of this structure always result in a decrease of this lytic activity.

3. The optical isomers of α-amino-acids are equally effective in causing lysis.

4. There is a correlation between the lytic and the bactericidal powers of the amino-acids and their derivatives.

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