

Bacitracin A: Structure-activity relationship

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Abstract. The single imidazole nucleus of L-histidine residue in bacitracin-A seems to be important for the anti-bacterial activity of the molecule, since iodination, carboxymethylation and coupling of diazobenzene sulphonic acid to the histidine residue in the antibiotic caused 90-94% loss of antibacterial activity of the antibiotic. In contrast, the bacitracin sulphone and sulphoxide derivatives are as active as the parent antibiotic.

Keywords. Bacitracin-A; chemical modification; iodination; carboxymethylation; diazotization.

Introduction

Previous investigations from this laboratory (Rambhav, 1981) on bacitracin-A have revealed that (i) the single chain amino group of D-ornithine residue is necessary for the anti bacterial activity on the molecule and (ii) the α - and γ -carboxyl groups of D-asparagine and D-glutamic acid residues of the molecule are not required for the antibacterial activity of the antibiotic. The present study deals mainly with the effect of iodination, carboxymethylation and coupling of diazobenzene sulphonic acid moiety to the imidazole group of the single L-histidine residue of the antibiotic on the antibacterial activity of the molecule. The oxidation of the sulphur atom in the thiazoline ring of bacitracin-A to the level of sulphoxide and sulphone on the antibacterial activity is also discussed.

Materials and methods

Bacitracin (58.4 units/mg, Calbiochem, Los Angeles, California, USA) was a gift from Prof. L. K. Ramachandran. All reagents and solvents obtained from commercial sources were purified as and when necessary. The diazobenzene sulphonic acid was prepared from sulphonilic acid (Pauly, 1904).

All colorimetric measurements were carried out with a Systronics double cell colorimeter, type 102 or with a ELICO photoelectric colorimeter, CL-20. Spectrophotometric measurements were done with a Toshniwal Spectrophotometer. The antibiotic content in bacitracin derivatives was based on weight, and where necessary checked by Pauly's reaction for imidazole group (Pauly, 1904). Diazo-

benzene sulphonic acid groups were estimated both by the determination of the total acid groups (Fraenkel-Conrat and Cooper, 1944) of the original and modified antibiotic; and by spectral analysis (Tabachnick and Sobotka, 1960).

Iodine content in the iodobacitracin was estimated by the alkali fusion method (Kendall, 1914). The extent of carboxymethylation of bacitracin was ascertained by titrating a known amount of the derivative against a standard alkali using Phenolphthalein indicator. Antibacterial activity of modified bacitracins was determined using two Gram-positive organisms, *Streptococcus faecalis* (ATCC 8043) and *Staphylococcus aureus* (NCIM 2079) as before (Rambhav, 1981).

Results

Preparations of modified bacitracins

Iodobacitracin: Bacitracin (30 mg) was dissolved in water (1.0 ml) and cooled to 0-4°C in an ice bath. The pH of the solution was adjusted to 5.5 with the addition of a few drops of acetic acid. The solution was treated with the iodine solution (0.1 ml, 0.1M I₂ in 0.5M KI) at 5 min intervals and stirred well in cold. A total of 1.0 ml of the reagent was added, maintaining the pH around 5.5 and left in the cold overnight. The contents were diluted to about 30 ml with cold acetone, mixed well and left in cold for about 2 h. The product was isolated by filtration and washed with a small quantity of cold acetone and finally dried under vacuum over sodium hydroxide pellets. The yield was 16 mg and the iodine content was 0.82 μmol/mol of antibiotic.

Carboxymethyl bacitracin: An aqueous solution of bacitracin (30 mg in 1.0 ml, adjusted to pH 5.5 with acetic acid) was treated with 0.1M aqueous solution of iodoacetate. A total of 2.0 ml of the reagent was added during the period of 1 h in the cold, maintaining the pH around 5.5. The reaction mixture was left in the cold overnight and later the product was precipitated by the addition of nearly 30 ml of cold acetone. The product was isolated by filtration. The yield was 19.8 mg and the extent of carboxymethylation was 78%.

Coupling of bacitracin with diazobenzene sulphonic acid

An aqueous solution of bacitracin (30 mg in 1.0 ml) was cooled to 0°C in an ice bath and the pH of the solution was lowered to 6.5 by the addition of a drop of dilute acetic acid. The reaction was initiated by the addition of the freshly prepared diazobenzene sulphonic acid (0.5 ml, 0.05 mmol per ml). A total volume of 4.0 ml of reagent was added during a period of 1 h in the cold with continuous stirring and maintaining the pH around 6.5. Later the reaction was allowed to proceed in the cold for about 18 h. The reaction mixture was then diluted with cold acetone to about 40 ml to precipitate the product. The product was isolated by filtration. The yield was 22 mg.

Bacitracin sulphone

To 30 mg of bacitracin in 1.0 ml of water, was added a few drops of dilute acetic acid to lower the pH to 6.0-6.2 and cooled to 0-5°C. A solution of 16 mg of

Chromatographic homogeneity of bacitracin derivatives

All modified bacitracins were chromatographed (descending chromatography in n-butanol: acetic acid: water (3:1:1), and gave single spots with ninhydrin reagent spray. The R_f values of the modified bacitracins are shown in table 1,

Table 1. Mobilities (R_f values) of Bacitracin and its derivatives

Sample	Solvent (n-butanol:acetic acid:water; 3:1:1)
Bacitracin	0.55
Iodo-bacitracin	0.48
Carboxymethyl-bacitracin	0.37
Diazobenzene sulphonic acid coupled bacitracin	0.60
Bacitracin-sulphone	0.16
Bacitracin-sulphoxide	0.25

Antibacterial activity

The results on the antibacterial activity of bacitracin and its derivatives are shown in table 2. The 50% inhibitory concentrations ($I_{50\%}$) for bacitracin in the assay, using *S. faecalis* and *S. aureus*, were 0.4 and 2.0 $\mu\text{g/ml}$, respectively. Iodination, carboxymethylation and coupling of diazobenzene sulphonic acid moiety to the histidine residue resulted in 90-94% loss of antibacterial activity. Bacitracin sulphone and sulphoxide derivatives, in contrast, were 84-97% as active as bacitracin.

Table 2. Antibacterial Activity of Bacitracin and Its Derivatives

Sample	<i>S. faecalis</i>		<i>S. aureus</i>	
	$I_{50\%}$ ($\mu\text{g/ml}$)	Activity (%)	$I_{50\%}$ ($\mu\text{g/ml}$)	Activity (%)
Bacitracin	0.4	100	2.0	100
Iodo-bacitracin	10.0	4	25.0	8
Carboxymethyl-bacitracin	8.5	5	28.0	7
Diazobenzene sulphonic acid coupled bacitracin	10.5	4	20.0	10
Bacitracin-sulphone	0.4	93	2.2	91
Bacitracin-sulphoxide	0.4	97	2.4	84

The $I_{50\%}$ values represent the average of three experiments.

Discussion

Bacitracins are closely related mixtures of bactericidal polypeptide antibiotics obtained from *Bacillus subtilis*. Bacitracins are active against numerous Gram-positive bacteria. The type of modifications studied in the present investigation are iodination, carboxymethylation and coupling of diazobenzene sulphonic acid residue to the single histidine residue in the antibiotic. The sulphur atom in the thiazoline ring of the molecule was also oxidized and the effects on its antibacterial activity are studied.

Bacitracin is stable only in the pH range 4.0-6.0. Hence all chemical modifications performed on the molecule have been carried out between pH 4.0-6.0, to ensure that stability of the rest of the molecule is unaltered. The property of insolubility of bacitracin in acetone was used to precipitate the derivatives from the reaction mixture. The purity of the derivatives was ascertained by paper chromatography. The degree of modification has been provided based on direct and/or indirect methods. A study of the antibacterial activity has revealed the essentiality of the intact basic imidazole nucleus of histidine residue, since its iodination, carboxymethylation and coupling of diazobenzene sulphonic acid residue results in 90-94% loss of activity. On the otherhand, the sulphur atom in the thiazoline ring of the molecule appears to be unimportant, atleast as regards antibacterial activity, since bacitracin sulphone and sulphoxide derivatives are as active as the unmodified antibiotic.

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