REQUIREMENT OF DIVALENT METAL IONS FOR BACITRACIN ACTIVITY

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ABSTRACT

ADLER, RICHARD H. (University of California, Los Angeles) AND JOHN E. SNOKE. Requirement of divalent metal ions for bacitracin activity. *J. Bacteriol.* **83**:1315-1317. 1962.—The antibiotic activity of bacitracin towards *Staphylococcus aureus* is completely suppressed by ethylenediaminetetraacetate. The conclusion that bacitracin activity requires the presence of metal ions is supported by the demonstration that the addition of a number of divalent ions reverses the effect of ethylenediaminetetraacetate. The most active of these ions, cadmium, manganese, and zinc, directly enhance the activity of bacitracin.

The antibiotic bacitracin has been known for a number of years (Johnson, Anker, and Meleny, 1945). Although considerable attention has been directed toward the elucidation of the chemical structure of bacitracin (Abraham, 1957), there have been few reports concerned with the biological properties of this polypeptide antibiotic. The observation that small amounts of ethylenediaminetetraacetate (EDTA) completely suppressed bacitracin activity led us to the conclusion that the presence of certain divalent metal ions is necessary for the antibiotic activity. Evidence supporting this conclusion is presented in this report. Weinberg (1959) had previously reported on the enhancement of bacitracin activity by zinc salts, and to a lesser degree by salts of cadmium, mercury, aluminum, and cobalt. We, however, find that mercury, aluminum, and cobalt are ineffective, and the activity of zinc is surpassed by both manganese and cadmium salts.

MATERIALS AND METHODS

Staphylococcus aureus (ATCC 10537) was used as the test organism. The determination of bacitracin activity was carried out according to the turbidimetric method of Darker et al. (1948). To

100 ml of 0.5% peptone, 0.4% yeast extract, and 0.3% beef extract was added 1.0 ml of a culture of *S. aureus* (18 to 24 hr). To 4.5 ml of this inoculated medium was added 0.5 ml of the assay sample, and the mixture was incubated at 38 C for 4.5 hr. The optical density was then measured in a spectrophotometer at 620 m μ using cylindrical cells (15 by 95 mm). Bacitracin, which was obtained from Nutritional Biochemicals Corp., contained 70 units per mg.

RESULTS AND DISCUSSION

As part of a program concerned with the biosynthesis of bacitracin, it was necessary to measure the activity of the antibiotic in the presence of bacterial extracts. The complete suppression of bacitracin activity by certain extracts, and the subsequent investigation of the factors present in the extracts, led to the demonstration that EDTA abolishes the antibiotic activity of bacitracin.

The effect of various concentrations of EDTA on the growth of *S. aureus* was tested in the presence of different amounts of bacitracin. Bacitracin markedly inhibits the growth of *S. aureus*, and small concentrations of EDTA completely abolish this inhibition (Fig. 1). Although EDTA, at concentrations of 1×10^{-3} M and greater, inhibits the growth of *S. aureus*, lower concentrations, which are effective in overcoming bacitracin activity, have little if any direct effect on *S. aureus* growth.

In view of the chelating properties of EDTA, it appeared likely that the effect of EDTA in suppressing bacitracin activity was due to its combination with ions. Accordingly, various metallic ions were tested to determine whether they might reverse the effect of EDTA. In Table 1, the various ions which were active are listed in decreasing order of their activity. These ions, which are active in reversing the effect of EDTA, had no demonstrable effect on the growth of *S. aureus* when tested at a concentration of $1 \times$

FIG. 1. Effect of EDTA and bacitracin on the growth of *Staphylococcus aureus*. The amount of bacitracin present in the experiments was (○) zero, (△) 0.2 units, (●) 0.4 units, and (□) 0.8 units.

TABLE 1. Effect of EDTA and metal ions on bacitracin activity*

Additions	Optical density	
	I	II
None.....	0.342	0.342
Bacitracin.....	0.147	0.114
Bacitracin, EDTA.....	0.328	0.320
Bacitracin, EDTA, Cd ⁺⁺	0.056	0.035
Bacitracin, EDTA, Mn ⁺⁺	0.081	0.056
Bacitracin, EDTA, Zn ⁺⁺	0.148	—
Bacitracin, EDTA, Pb ⁺⁺	—	0.148
Bacitracin, EDTA, Co ⁺⁺	—	0.150
Bacitracin, EDTA, Ni ⁺⁺	—	0.162
Bacitracin, EDTA, Al ⁺⁺⁺	—	0.212
Bacitracin, EDTA, Fe ⁺⁺	0.234	—

* *Staphylococcus aureus* was grown as described under Materials and Methods. The amount of bacitracin present was 0.2 units while the concentration of EDTA was 1×10^{-5} M. The various salts that were added were present at a concentration of 1×10^{-5} M.

10^{-5} M and in the absence of bacitracin. It should be noted that cadmium and manganese ions not only reverse the effect of EDTA, but also, when these ions are added, the inhibition of *S. aureus* growth is greater than in the absence of both ions and EDTA. When tested under the conditions shown in Table 1, the following ions were inac-

tive in restoring bacitracin activity: Hg⁺⁺, Ba⁺⁺, Ca⁺⁺, Fe⁺⁺⁺, Cu⁺⁺, and Mg.

Although the ions listed in Table 1 are effective in reversing the suppression of bacitracin activity by EDTA, it is not evident whether these ions are capable of exerting a direct effect on the activity of the antibiotic. If the activity of bacitracin, measured in the absence of EDTA, is the result of a specific ion which is present in one or more of the components of the medium, the effect of the ions listed might be due to their displacing this specific ion from the EDTA which was added. Consequently, the various ions were tested for their effect on bacitracin activity in the absence of EDTA. When tested at a concentration of 4×10^{-6} M, only salts of cadmium, manganese, and zinc were capable of enhancing the activity of bacitracin. Lead, cobalt, nickel, aluminum, and iron salts, which were effective in reversing the action of EDTA, did not enhance bacitracin activity when tested in the absence of EDTA. In Fig. 2 are shown the effects of different concentrations of cadmium, manganese, and zinc ions. It is evident that, of these ions, cadmium is by far the most active.

The above results would appear to demonstrate that the antibiotic activity of bacitracin is

FIG. 2. Effect of various metal ions on bacitracin activity. The amount of bacitracin present in the experiment was 0.10 units. The metal ion added is given by (□) zinc, (●) manganese, and (△) cadmium. The growth of *Staphylococcus aureus* in the absence of bacitracin is represented by the dashed line at the top of the figure.

dependent upon the presence of certain divalent cations. However, it was necessary to consider the unlikely possibility that the effect of the cation was to protect the antibiotic from being destroyed. This was tested by growing *S. aureus* for 4 hr in the presence of 0.2 units of bacitracin and 1.2×10^{-5} M EDTA, removing the cells by centrifugation, and then assaying the growth medium for bacitracin. The assay of the growth medium for bacitracin was carried out after the medium was reincubated with fresh *S. aureus*, and Mn ions were added so that the concentration was 3.6×10^{-6} M. The result of such an experiment revealed not only that the bacitracin in the medium was fully active, but also that if any bacitracin was taken up by the growing *S. aureus* the amount was negligible.

At the present time, the basis for the antibiotic action of bacitracin is unknown. Although the requirement for certain metal ions has been established, the role of the metal ion in activating the polypeptide is likewise unknown.

The conclusion of Weinberg (1959) that bacitracin activity requires the presence of certain metal ions is confirmed by the present study. However, we have been unable to show that mercury, cobalt, and aluminum salts directly activate bacitracin. The possibility that the re-

ported activity of these salts might be due to an active metal ion present as an impurity is difficult to evaluate since the concentration of these salts used was not reported. In contrast to Weinberg's conclusions that bacitracin activity was enhanced most strongly by zinc ion, we find both cadmium and manganese to be more active than zinc.

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