ON THE INHIBITION OF HEN EGG-WHITE LYSOZYME ACTIVITY BY AMINOGLYCOSIDIC ANTIBIOTICS

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SUMMARY. Lytic activity of hen egg-white lysozyme towards bacterial cells of *Micrococcus lysodeikticus* was pH-dependent inhibited by several aminoglycosidic antibiotics, the structure of which is related to the saccharidic substrates of the enzyme.

Inhibition extent suggests the role of the positive charges of this type of antibiotics on the mechanism of lysozyme activity inhibition.

Lysozyme is a glycosidase which hydrolyses N-acetyl-hexosaminidic linkages in chitin, in the β(1→4)-linked linear chain polymer of N-acetylglucosamine in bacterial cell walls and in other various high and low molecular weight compounds related to these polymers (1,2). It has been also observed that several low molecular weight saccharides inhibit the digestion of *Micrococcus lysodeikticus* cell walls by lysozyme, presumably by its binding to the active site of the enzyme (3-6). On the other hand, some aminoglycosidic antibiotics have been demonstrated to increase the renal content of soluble lysozyme (7,8).

In regard to this inhibition, it has to be emphasized that some antibiotics bind to hemoglobin, carbonic anhydrase, serum albumin and other proteins (9-11). It is not well established whether the binding of antibiotics affects the function of these proteins; however, this protein binding affects the antimicrobial activity of antibiotics.

Taking into account the structural resemblance of some amino - glycosidic antibiotics to the low molecular weight saccharides derived from mucopolysaccharides, we have investigated extensively the effect of a series of these antibiotics on the lytic activity of hen egg - white lysozyme towards bacterial cell walls at different pH and ionic strength values.

MATERIALS AND METHODS

Crystalline hen egg-white lysozyme and *Micrococcus lysodeikticus* dried cells were obtained from Sigma Chem.Co (St.Louis, USA).

Dihydrostreptomycin, neomycin B, gentamicin C_{1a} and kanamycin A

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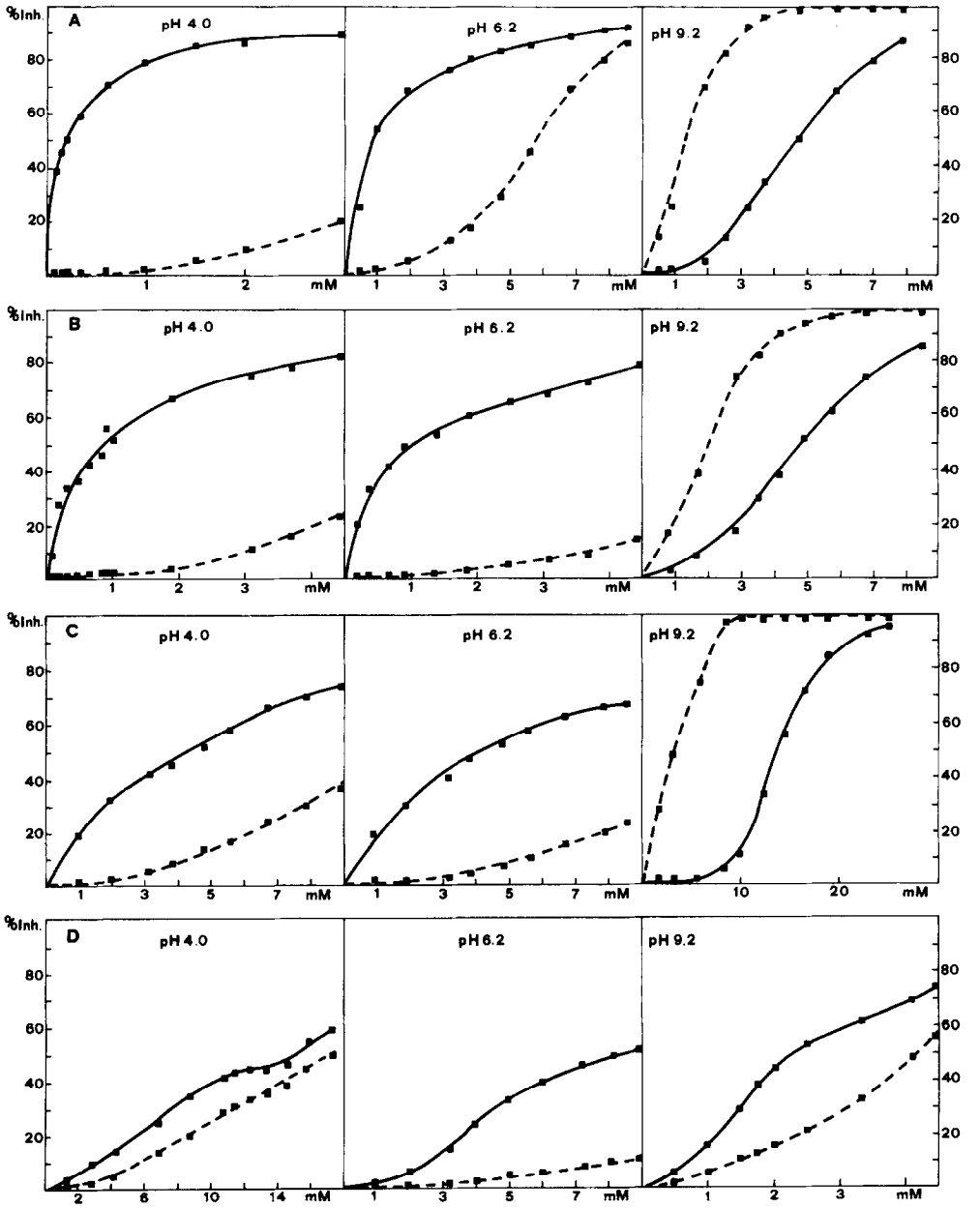


Figure 1. Hen egg-white lysozyme inhibition patterns at different pH values produced by: A) neomycin B

B) gentamicin C_{1a}

C) kanamycin A

D) dihydrostreptomycin

(——) Total inhibition caused by the antibiotic sulphates.

(----) Ionic strength inhibition by NaCl solutions corresponding to the ionic strength of the antibiotic sulphates.

were specially prepared and purified as sulphates for this research by Antibióticos,S.A.(León, Spain).

Suspensions of *Micrococcus lysodeikticus* (6.2 mg/30 ml) dried cells were prepared in buffer solutions at different pH values. Hen egg-white lysozyme (0.13 mg/ml) in the same buffers was used for these studies. Inbition assays by the antibiotics were performed by the final addition of 20 µl of enzyme solution to 1 ml of suspension of bacterial cells plus 50 µl of the corresponding antibiotic solution in the same buffers at the various concentrations studied; activities were expressed as percentages of the enzyme activity showed in the absence of inhibitor. Enzyme inhibition by ionic strength was checked using the same solutions and the ionic strength (I) was increased by adding NaCl to the mixture of reaction. Activities were refered to the assay in the absence of salt.

Protein concentrations were measured by the absorbance at 280 nm with a Varian Techtron model 635D digital spectrophotometer. A molar absorbance coefficient for hen egg-white lysozyme at 280 nm, ε=36500, was used throughout (12).

Buffer solutions used were: sodium acetate-HCl, pH 4.0 (I=0.1); glycine-NaOH, pH 9.2 (I=0.04); potassium phosphate, pH 6.2 (I=0.067).

RESULTS AND DISCUSSION

Figure 1 shows the different inhibition patterns of hen egg-white lysozyme activity by various aminoglycosidic antibiotics at pH 4.0, 6.2 and 9.2. Percentages of inhibition as $(1-v_I/v_O).100$ are plotted versus antibiotic concentrations, where v_O and v_I are, respectively, the initial velocities in either absence or presence of inhibitor.

Since the lysozyme activity is strongly dependent on ionic strength (13) and the aminoglycosidic antibiotics were used as sul - phates, it was of interest to obtain evidence on the enzyme inhibition caused by NaCl at concentrations having the same ionic strength than those produced by the different concentrations of the antibiotic salts.

The concentrations at which the antibiotics caused 50% inhibition at pH 4.0 and 6.2 are lower for neomycin B than for the other three antibiotics tested; gentamicin C_{1a} inhibits 50% of the control activity at concentrations very close to those of neomycin B that produce the same extent of inhibition. The concentration of antibiotics necessary to yield 50% inhibition, at both pH values, can be arranged according to neomycin B < gentamicin C_{1a} < kanamycin A < dihydrostreptomycin (see Table 1). The extent of inhibition is always greater at pH 4.0 than at pH 6.2, except for dihydrostreptomycin.

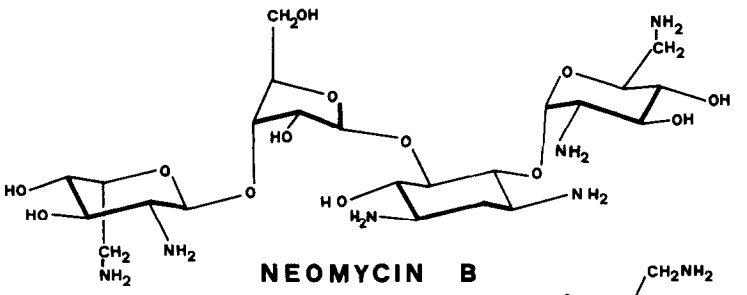
TABLE 1. Inhibition of hen egg-white lysozyme activity on *M. lysodeikticus* cells by various aminoglycosidic antibiotics.

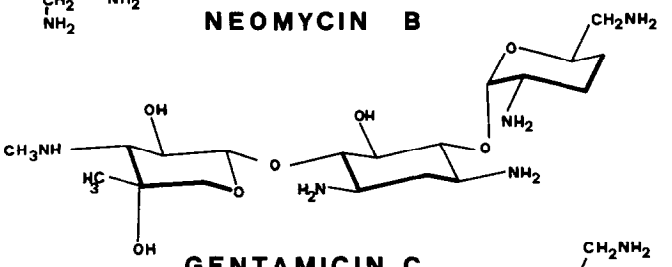
Antibiotic	Concentration (10^{-3}M) for 50% inhibition of cell lysis		
	pH 4.0	pH 6.2	pH 9.2
Neomycin B	0.2	0.8	4.8
Gentamicin C_{1a}	0.8	0.9	5.0
Kanamycin A	4.0	4.2	14.0
Dihydrostreptomycin	14.0	8.3	2.3

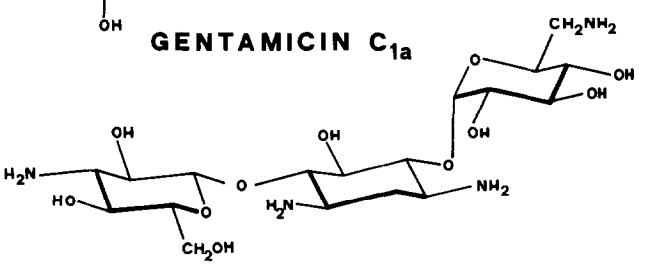
The above results do not prove undoubtedly the competitive nature of this inhibition. On the other hand, it has been quite difficult to obtain valuable graphic representations on the nature of the inhibition because of the special characteristics of the substrate and the lyso - zyme activity determination (14,15). Nevertheless, due to the struct - ural similarities among the aminoglycosidic antibiotics and the lyso - zyme substrates (figure 2) it is very likely that the inhibition could be competitive and that these antibiotics bind to the enzyme at the same site as the substrates. It is interesting to note that neomycin B, having a four-cycle structure, is a stronger inhibitor of lysozyme than the three-cycle aminoglycosidic antibiotics, gentamicin C_{1a}, kanamycin A and dihydrostreptomycin, at identical concentrations. However, it has been described that the degree of inhibition of lysozyme activity by oligomers of N-acetylglucosamine do not increase with chain length beyond chitotriose (16,17). Thus, whether the higher inhibition of lyso zyme activity by neomycin B could be due to chain length remains to be clarified.

The concentration of antibiotics necessary for 50% inhibition, at pH 9.2, changes according to the order dihydrostreptomycin < neomycin B < gentamicin C_{1a} < kanamycin A. Thus, the order at which 50% is attain- ed by the various antibiotics, at pH 9.2, remains the same as that for pH 4.0 and pH 6.2, except for dihydrostreptomycin that at these pH values was the weakest inhibitor whereas at pH 9.2 became the strong- est inhibitor of all antibiotics studied.

These results can be interpreted taking into account that posi- tive charges of the aminoglycosides play an important role in the







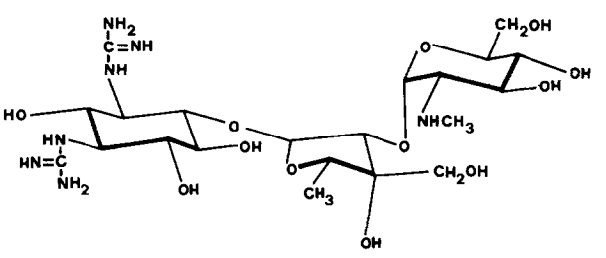


Figure 2. Chemical structure of the various aminoglycosidic antibiotics used in the inhibition assaya of the hen egg-white lysozyme activity.

inhibition of lysozyme activity. At pH 9.2, dihydrostreptomycin is the strongest inhibitor and its two guanido groups have to be fully pro - tonated whereas the other antibiotics do not possess such basic groups and, consequently, they cannot be so positively charged at this pH value. On the other hand, the most potent inhibitor of all these amino glycosidic antibiotics, at pH 4.0 and pH 6.2, is neomycin B followed

by gentamicin C_{1a}, kanamycin A and dihydrostreptomycin, according to the number of positive charges they bear.

Preliminary binding studies of aminoglycosidic antibiotics to hen egg-white lysozyme show the existence of an interaction protein-antibiotic (14). Further binding studies are now in progress.

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