INTERACTION OF AMINOGLYCOSIDES AND OTHER ANTIBIOTICS WITH ACTIN

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(Received for publication November 20, 1978)

Skeletal muscle actin was found by centrifugation, turbidity, and viscosity measurements to form polymers upon addition of aminoglycosides, viomycin, polymyxin B, and tetracycline. A linear relationship was observed between the amount of actin polymerization and the number of primary amino groups on the aminoglycoside antibiotics except kanamycin. Of the antibiotics studied, neomycin was most efficient in actin polymerization. Polymerization of actin was not significantly induced by kasugamycin, chloramphenicol, erythromycin, benzylpenicillin, angustmycin A, formycin, actinomycin D, and mitomycin C.

Aminoglycosides and viomycin were demonstrated to inhibit the acto-HMM Mg^{2+}-ATPase reaction but did not significantly affect HMM Mg^{2+}-ATPase activity. It was found by equilibrium dialysis that [^{3}H]dihydrostreptomycin bound to actin.

The aminoglycoside and viomycin groups of antibiotics have the potential to cause nephrotoxicity, ototoxicity, and neuromuscular blockade. For the purpose of establishing the biochemical mechanism of side effects of antimicrobial antibiotics, we have studied the effects of antibiotics on mammalian cell components, and found that several aminoglycosides and viomycin interact with tubulin and microtubules[1]. We have further observed interactions of aminoglycosides, viomycin, polymyxin B, and tetracycline, which are associated with neuromuscular paralysis (*cf.* a review[15]), with muscle actin. The results are presented in this publication.

Materials and Methods

Antibiotics used were streptomycin (Meiji Seika Kaisha), kanamycin (Meiji Seika Kaisha), neomycin (Fradiomycin, Takeda Chemical Industries), gentamicin C_1, C_{1a} and C_2 (Schering Research Division), kasugamycin (Institute of Microbial Chemistry), polymyxin B (P-L Biochemicals), chloramphenicol (Sankyo Company), tetracycline (Lederle, Japan), erythromycin (Shionogi and Company), benzylpenicillin (Meiji Seika Kaisha), formycin (Institute of Microbial Chemistry), angustmycin A (Meiji Seika Kaisha), mitomycin C (Kyowa Hakko Kogyo), and viomycin (Pfizer Taito Co.).

[^{3}H]Dihydrostreptomycin sulfate (3 Ci/mmole) was purchased from the Radiochemical Centre, Amersham, England.

Heavy meromyosin was prepared by tryptic digestion of myosin isolated from rabbit skeletal muscles, as described by YAGI *et al.*[2] Actin was isolated from acetone powder of rabbit skeletal muscle and purified by the method of SPUDICH and WATT[3]. G-Actin concentration was determined using the absorption coefficient $A_{1\%}^{290\,nm}$ of 0.63[4], and HMM content by LOWRY's method[5].

Actin polymerization:

Polymerization of actin was followed by measuring protein content in centrifugal supernatant, turbidity and viscosity of actin solution, incubated with antibiotics.

The centrifugal analysis was carried out by the technique of ORIOL-AUDIT[6], using a buffer (2 mM Tris-HCl, pH 7.6, 0.2 mM $CaCl_2$, 0.1 mM ATP, and 1 mM 2-mercaptoethanol). The results were

Abbreviation: HMM, heavy meromyosin.

corrected for appropriate blanks.

The antibiotic in a buffer (2 mM Tris-HCl, 0.2 mM $CaCl_2$ and 0.1 mM ATP, pH 7.6) was added to G-actin (1 mg/ml) in the same buffer. After incubating the mixture for 30 minutes at room temperature, the viscosity was measured with a 1-ml Ostwald type viscometer at 25°C (outflow time for water was 30 seconds). The relative viscosity η_r was determined as the ratio of the flow time of samples to that of water. The specific viscosity η_s was $\eta_r - 1$. The turbidity of the mixture was measured at 350 nm.

Assay of Mg^{2+}-ATPase of HMM and acto-HMM:

The ATPase activity was determined by the procedure described previously[7].

Equilibrium dialysis:

The binding of [^{3}H]dihydrostreptomycin to actin was measured by equilibrium dialysis, as described previously[8]. The molecular weight of G-actin was assumed to be 4.3×10^4, and that of HMM 3.4×10^5.

Results

Polymerization of Actin by Antibiotics

Addition of aminoglycosides, polymyxin B, viomycin, and tetracycline was observed by the centrifugal analysis method to induce polymerization of actin. Fig. 1 shows the percentage of actin polymerization as a function of the concentration of the antibiotics. Gentamicins C_1, C_{1a} and C_2, neomycin, and polymyxin B at concentrations of 0.1 mM caused actin polymerization up to 90%. The polymerization took place to a significant extent even at concentrations of 0.02 mM. Kanamycin, streptomycin, viomycin, and tetracycline exhibited lesser polymerizing activity. Kasugamycin, erythromycin, chloramphenicol, benzylpenicillin, angustmycin A, formycin, actinomycin D, and mitomycin C did not significantly induce polymerization of G-actin at antibiotic concentrations of 0.5 mM. At a constant antibiotic concentration of 0.02 mM, a linear relationship was observed between the yield of actin polymerization and the number of primary amino groups in aminoglycoside molecules, except for kanamycin (Fig. 2). The tendency of the antibiotics to polymerize actin was also de-

Fig. 1. Percentage of actin polymerization as a function of antibiotic concentration.

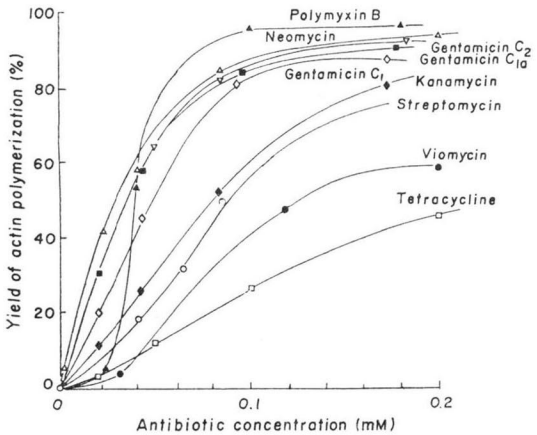


Fig. 2. Relationship of the number of primary amino groups of aminoglycoside antibiotics to the yield of actin polymerization.
1. streptomycin, 2. gentamicin C_1, 3. gentamicin C_{1a}, 4. gentamicin C_2, 5. neomycin, 6. kanamycin

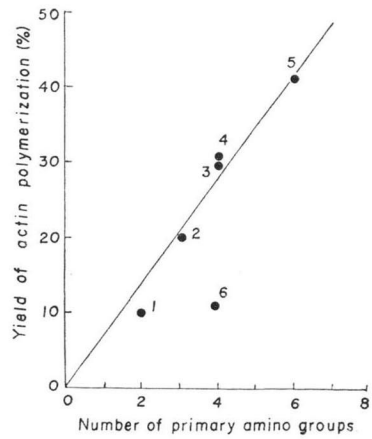


Fig. 3. Turbidity change of actin induced by antibiotics.

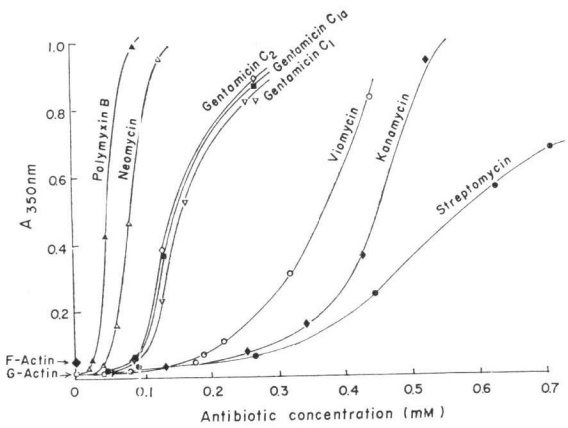
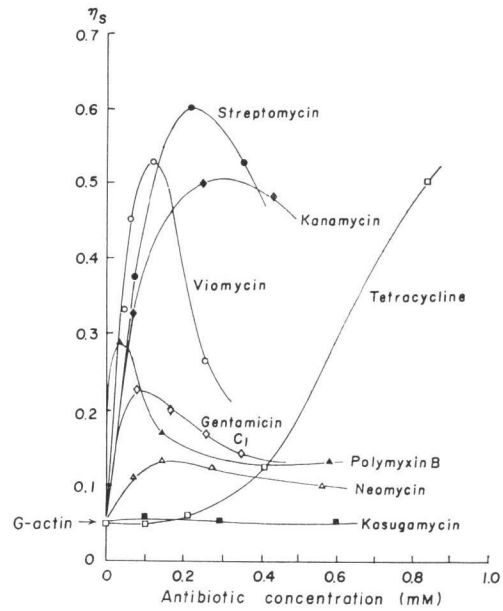


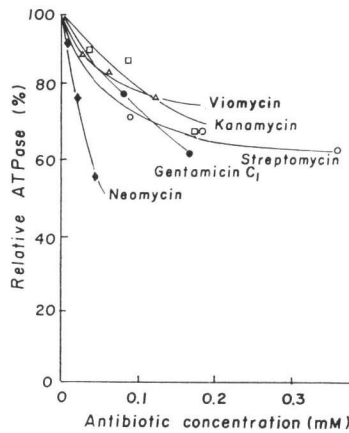
Fig. 4. Viscosity of actin polymers induced by various antibiotics.



monstrated by the turbidity method (Fig. 3). The turbidity of salt-induced F-actin was much lower than that of actin polymers induced by these antibiotics. This seemed to imply that the antibiotic-induced actin polymer contained bundles of F-actin filaments.

The viscosity of G-actin solutions was increased by addition of aminoglycosides, polymyxin B, viomycin, and tetracycline (Fig. 4). With most of the antibiotics, except tetracycline, maximal values of the specific viscosity η_s exist: *i.e.* η_s increased to maxima at certain antibiotic concentrations and then decreased as the antibiotic concentrations increased. The decrease of viscosity at high antibiotic concentrations was ascribed to parallel aggregate formation. The values of η_s in the presence of gentamicin C_1 and neomycin were lower than those with streptomycin, kanamycin, and viomycin, although the percentage of sedimentable actin was higher with the former group of antibiotics than with the latter.

Fig. 5. Effects of aminoglycoside antibiotics and viomycin on acto-HMM Mg^{2+}-ATPase reaction.
100%=0.84 μmol/min/mg HMM.
actin 1.0 mg/ml, HMM 0.5 mg/ml.



Inhibition of Acto-HMM Mg^{2+}-ATPase Reaction by Aminoglycosides and Viomycin

As illustrated in Fig. 5, the acto-HMM ATPase activity was blocked by streptomycin, kanamycin, neomycin, gentamicin C_1 or viomycin at antibiotic concentrations that produced polymerization of actin. Neomycin was most efficient among the antibiotics tested; the degree of inhibition was *ca.* 50% at 0.05 mM neomycin while only 30~40% inhibition was observed in the presence of 0.2 mM of

the other antibiotics.

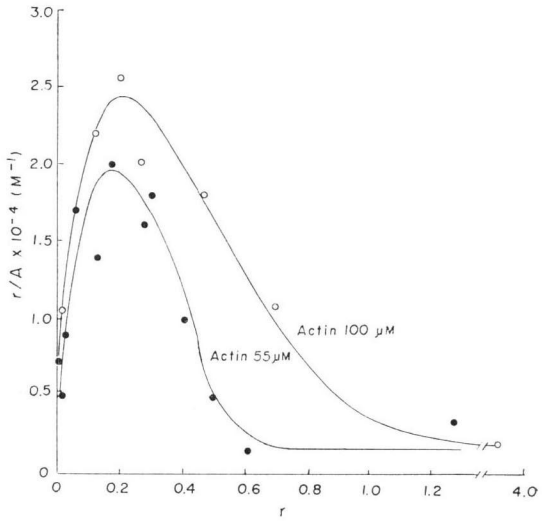
On the other hand, in a medium of low ionic strength HMM Mg^{2+}-ATPase was not significantly affected by any of the antibiotics examined at concentrations which actin aggregated (data are not shown).

Binding of [^{3}H]Dihydrostreptomycin to Actin

The association constant and stoichiometry for the binding were determined by equilibrium dialysis, using various concentrations of [^{3}H]dihydrostreptomycin and two concentrations of actin. SCATCHARD plots of equilibrium binding are presented in Fig. 6. The curves were open at the bottom, and moved upward for increasing actin concentrations.

Fig. 6. SCATCHARD plots for equilibrium binding of [^{3}H]dihydrostreptomycin to actin. The buffer used contained: 2 mM Tris-HCl, pH 7.6, 0.2 mM $CaCl_2$ and 1 mM ATP.

A is the molar concentration of free antibiotic, and r is the number of molecules of bound antibiotic per actin monomer.



The analysis of the results appeared to be difficult. However, according to the guideline of CANN[9], this system seemed to correspond to the ligand-facilitated mechanism of protein polymerization. The shape of the SCATCHARD plots was reminiscent of nonassociation systems characterized by binding to multiple sites with positive cooperativity. In the case of ligand-facilitated polymerization, the SCATCHARD plots were dependent upon protein concentration, and the intercept with the ordinate gave an intrinsic binding constant. Thus, the results suggested that actin monomer may possess one intrinsic binding site with an association constant of 5×10^3 M^{-1} and several extraneous binding sites with less affinity for dihydrostreptomycin.

Discussion

The biological significance of the interaction of aminoglycosides and the other antibiotics with actin remains to be determined. However, the interaction of the antibiotics with actin seems to parallel their neuromuscular blocking activity (*cf.* a review[15]). Aminoglycosides, viomycin, polymyxin B, and tetracycline are known to cause neuromuscular blockade, and have been found in the current experiments to interact with actin. In contrast, chloramphenicol, erythromycin, and benzylpenicillin, lacking in the activity of neuromuscular paralysis, have been observed not to interact with actin. Neomycin is most efficient both in actin polymerization and in neuromuscular blockade. The current studies concern the interaction of the antibiotics with muscle actin. However, the results may be extended to actin of other cells, since several investigations have shown that microfilaments or actin-like proteins of non-muscle cells or tissues possess characteristics similar to muscle actin[10~14,16]. If so, the observed interaction of aminoglycosides and the other antibiotics with actin may be related to their side effects. Aminoglycosides primarily prevent the prejunctional release of acetylcholine and also depress post-junctional sensitivity to the humoral agent (see a review[15]). BERL *et al.*[16] have suggested that an actomyosin-like protein may function in the release of transmitter agent at synaptic endings. If so, the antibiotics associated with neuromuscular blockade may interfere with the release of transmitter material at neuromuscular junctions.

G-Actin is transformed into a fibrous polymer by the addition of neutral salts at the physiological concentration: *i.e.* KCl 0.1 M. The antibiotic-induced actin polymers, observed in the current experiments, exhibit characteristics different from those of salt-induced F-actin, as demonstrated by the turbidity and viscosity methods. This seems to be related to the inhibition of acto-HMM Mg^{2+}-ATPase activity by the antibiotics.

Recently ORIOL-AUDIT[6] has found that polymerization of actin is induced by polyamines, and has observed a linear relationship between the yield of actin polymers and the chain length of polyamines. We have observed here that the number of primary amino groups in various aminoglycoside antibiotics parallels their actin-polymerizing activity.

We have reported that adriamycin and daunorubicin, anthracycline antibiotics, inhibit HMM Mg^{2+}-ATPase, and suggested a relationship to their cardiotoxicity[7]. All the antibiotics examined in the present experiments fail to block HMM Mg^{2+}-ATPase, indicating the inhibition of HMM Mg^{2+}-ATPase by adriamycin and daunorubicin is specific to these antibiotics.

Acknowledgements

The authors express their deep thanks to Dr. HAMAO UMEZAWA for his kind advice and cooperation in the present study.

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