Kanamycin A

An assignment by:
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Kanamycin A is an aminoglycoside antibiotic obtained from Streptomyces kanamyceticus and was isolated from Japanese soil. It was first described in 1957 by Y.Okami and H.Umezawa. Kanamycin A is the main component of commercially available kanamycin, which still contains kanamycin B and C.

2. Structure

The basic unit of kanamycin is an aminocyclitol, the 2-deoxy-D-streptamin (2-DOS, in red), which is substituted at the 4- and 6-position. The three kanamycins are constitutional isomers that differ only in their number of amino and hydroxyl groups. In kanamycin A the 4-OH group of 2-DOS is associated with an aminoglucopyranose and the 6-OH group with a derivative of glucosamine (glucosamine: 2-Amino-2-desoxy-α/β-D-glucopyranose).

The IUPAC name for kanamycin A is therefore:

4 - (6-deoxy-6-amino-α-D-glucopyranosyloxy) - 6 - (3-deoxy-3-amino-α-D-glucopyranosyloxy) - 2-deoxy-D-streptamin

The empirical formula is thus: C_{18}H_{36}N_{4}O_{11}.

3. Physical and chemical properties

<table>
<thead>
<tr>
<th>property</th>
<th>value</th>
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</thead>
<tbody>
<tr>
<td>molar mass</td>
<td>484.50 g / mol</td>
</tr>
<tr>
<td>solubility</td>
<td>water soluble</td>
</tr>
<tr>
<td>colour</td>
<td>colourless</td>
</tr>
<tr>
<td>aggregate state</td>
<td>solid</td>
</tr>
<tr>
<td>(298,15K, 1atm)</td>
<td></td>
</tr>
</tbody>
</table>

Because of its amino and hydroxyl functionalities Kanamycin A is an alkaline, strong-polar and hygroscopic oligosaccharide. Within the biologically relevant pH range the amino groups are protonated (pK_a (NH_2) = ~ 4.3), consequently Kanamycin A exists as a salt.

Hazards: s-phrases: 22: do not breathe dust 23: do not breathe gas/fumes/vapour/aerosol 24: avoid contact with skin
4. Use

In 1957, during their tuberculosis research, the Japanese scientists Hamao Umezawa (1914-1986) and Y. Okami discovered the antibiotic kanamycin A. Umezawa discovered 12 antibiotics in total, 18 anti-cancer drugs and some enzyme inhibitors. In his honor, the “International Society of chemotherapy, infection and cancer” (ISC) named its “ISC award” the “Hamao Umezawa Award”. He was also honored by the Vatican for his work.

Kanamycin A is one of the aminoglycoside antibiotics like streptomycin or neomycin. All of them are tri- or tetrasaccharides, their other common characteristics are the streptamin component and the bactericidity.

<table>
<thead>
<tr>
<th>drug specifications</th>
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<tr>
<td>drug classification</td>
<td>antibiotic, aminoglycoside</td>
</tr>
<tr>
<td>prescription</td>
<td>yes</td>
</tr>
<tr>
<td>method of administration</td>
<td>injection: intramuscular injection or intravenous tablet: oral</td>
</tr>
<tr>
<td>LD₅₀</td>
<td>17 500 mg / kg (mice, orally)</td>
</tr>
<tr>
<td></td>
<td>&gt; 4000 mg / kg (rats orally)</td>
</tr>
<tr>
<td></td>
<td>&gt; 3000 mg / kg (rabbits, orally)</td>
</tr>
<tr>
<td>half life</td>
<td>2.5 hours</td>
</tr>
<tr>
<td>storage</td>
<td>desiccator, protection from direct sunlight, stable in solution: 2-8 °C, 12 months</td>
</tr>
</tbody>
</table>

Kanamycin A is commercially available as the main component of the active pharmaceutical agent Kanamycin, developed and launched by the Sigma Aldrich Corporation.

In human medicine Kanamycin A is used to treat bacterial infections of the eyelids, conjunctiva and cornea through kanamycin-sensitive bacteria. It’s applied as sulphate salt in form of eye drops and ointments (e.g. Kanamytrex®, Kana-Stulln®, Kan-Ophtal®). It is also used as a reserve antibiotic to treat multi-resistant tuberculosis and as a reserve antibiotic for the treatment of gastro-intestinal infections through kanamycin-susceptible pathogens in dogs and cats.
5. Mode of action

Kanamycin, including kanamycin A, acts in a bactericidal manner, since they can penetrate the bacterial cell wall by oxygen-dependent active transport and inhibit bacterial protein synthesis. They interact with the decoding site at nucleotide 1492 in the 16S-rRNA of the 30S subunit of the prokaryotic ribosome.

Mechanism of prokaryotic protein biosynthesis:
In the first step, the transcription, the so-called mRNA is encoded. The mRNA contains complementary copies of certain DNA segments and is build by base pairing. In the second step, the translation, this mRNA is decoded in the ribosome. In this purpose, loaded tRNA, whose anticydon is complementary to the just mentioned codon, is bound to the mRNA. The favored peptide is gained as peptide bonds are established between all identified amino acids.

In translation both parts of the prokaryotic 70S ribosome are involved: the 30S and the 50S subunit. Normally they exist separately, but during the translation they get together and form two functionally important regions, to which the tRNAs can bind:
- at the peptidyl site sits the tRNA with the growing protein chain
- at the aminoacyl point sits the tRNA with next amino acid to be added.

The translation begins as soon as an initiator tRNA binds to the start codon.
In bacteria, this initiator tRNA is always loaded with N-formylmethionine.
The complex between mRNA, 30S subunit and formylmethionine-tRNA is called initiation complex.

Effect of kanamycin:
The highly polar kanamycin A binds irreversibly to the 30S subunit and freezes the initiation complex, so that the mRNA and the protein can no longer be read and no longer be elongated.
This leads to the disintegration of polysomes (many ribosomes lining up at the mRNA to be transferred, during the protein biosynthesis) into useless monosomes.
On the one hand, therefore no more pathogenic proteins can be synthesized, on the other, bacteria die off, because protein biosynthesis is existential.

Because of the oxygen-dependent transport into the cell, Kanamycin only functions against aerobic pathogens.

It is particularly effective against aerobic gram-negative pathogens (Enterobacter, Pseudomonas, Actinobacter) and against mycobacteria (mycobacterium tuberculosis).

Kanamycin works only against the prokaryotic protein biosynthesis, because the prokaryotic 70S ribosome is different to the eukaryotic 80S ribosome.
6. Kanamycin resistance

One problem in treatment with kanamycin A is that some prokaryotic microorganisms are resistant to kanamycin. The most common reason is the existence of a so-called kanamycin nucleotidyltransferase. It prevents the activity of kanamycin, as it catalyzes the transfer of a nucleotide monophosphate to a hydroxyl group of the aminoglycoside. The nucleotidyltransferase is a dimer composed of α-helices and β-sheets. It binds by hydrogen bridge bonds, for example the adenine in ATP. Thereupon the glutamic acid site of the enzyme splits off a proton from the 4-hydroxyl group of kanamycin. In this process kanamycin A becomes an oxygen-nucleophile and attacks the α-phosphate group of the ATP, the two remaining phosphate groups of ATP are split off. This change of a functional group causes the kanamycin to be deactivated. The kanamycin nucleotidyltransferase was found for the first time in Staphylococcus aureus.

7. Side effects

The following side effects occur on the one hand from a binding affinity of kanamycin to other polar molecules, or by enrichment due to a pH gradient. Kanamycin is classified as:

- nephrotoxic: accumulation in the renal cortex can lead to kidney failure.
- ototoxic: accumulation in the endolymph of the inner ear can cause damage to the sensory cells and to the hearing.
- neurotoxic: kanamycin impedes the release of acetylcholine at the motor end plate.

Apart from that there is also an increased risk for pregnant women, as kanamycin can cross the placental barrier.

**Conclusion:** In recent years the use of kanamycin has decreased because it is more toxic than streptomycin and has the weakest antibacterial activity of amino glycosides in medical use.

8. Use as selection antibiotic

In addition to medical application Kanamycin is used in molecular biology as a selection antibiotic. Because it is toxic to bacteria and plants, transgenic plants and bacteria are equipped with additional resistance genes to kanamycin. The cultivation on kanamycin-containing medium allows selection of altered compared to native microorganisms or plants. This procedure has been criticised, because previously kanamycin-sensitive bacteria have become immune through horizontal gene transfer.
Example: “Amflora”
In March 2010 the European Commission allowed the cultivation of “Amflora”, a genetically modified potato developed by BASF. Their starch consist exclusively of amylopectin, so that the normally required extensive removal of the second starch polymer, amylase, can be dispensed with.

8. Synthesis

a) Chemical analysis

Kanamycin A is a derivative of streptamin with two glycosidic linkages. The building of glycosidic linkages is common, but the synthesis of 2-deoxystreptamin (2-DOS) is interesting!

2-DOS is an aminocyclitol.

The formation starts with a Diels-Alder-reaction between cyclopentadien and cylohexa-2,5-diene-1,4-dione (1,4-Benzooquinone) to molecule (1).

The Diels-Alder product is then reduced through a Luche reduction (hydrogenation) with NaBH$_4$ and CeCl$_3$ to the endiol (2). The endiol reacts with PdCl$_2$ and HCO$_2$NH$_4$ to an α,β-unsaturated ketone (3). This reaction starts with a protonation and is followed by dehydration and hydride-shift.

The double bond of the α,β-unsaturated ketone is now used for the formation of an epoxide (4) by H$_2$O$_2$. The exo-formation of the epoxide is aided by a Bürgi-Dunnitz-stabilisation and steric hindrance. Steric hindrance also inhibits the reaction of the second double bond. Afterwards the keto group is reduced to a hydroxyl group what leads to a product mixture because of an equilibrium of both structures.
Now a retro-Diels-Alder-reaction (vacuum, 80 degrees Celsius) yields intermediate (13) which is attached to the protecting group tert-Butyl(dimethyl)silane chloride (14) through nucleophilic addition. With NaN₃ an azide group adds to the epoxide which is opened (15).

Finally a new epoxide (16) is formed with mCPBA (in the opposite direction of TBDMS) and the epoxide is again opened by NaN₃.
To get 2-DOS, the azid groups have to be reduced by H₂/Pd/C, the protecting group has to be removed and the two glycosidic linkages have to be synthesized in acetalisations.

**b) Biosynthesis**

Kanamycin A is biosynthetically synthesized by Streptomyces kanamyceticus. Streptomyces kanamyceticus are gram-positive, aerobic, multicellular and rod-shaped. They belong to the phylum Actinobacteria and are found in the soil. However some of the bio synthetcal steps have not yet been declared safe! The biosynthesis route is a postulate. Assumptions could be made by comparisons with other biosynthesis and the functions of enzymes of similar composition.
Documented Intermediates: 2-DOS, paromamine, neamine and RIB

The skeletal structure:

Kanamycin A is formed in an enzyme-catalyzed reaction of the metabolite glucose-6-phosphate in a process by which the glycosidic group is changed and binds to sugar residues. Kanamycin B and C are intermediates. This explains why kanamycin-based drugs include all three components.
9. Bibliography

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