STUDIES ON ADENYLYLSTREPTOMYCIN, A PRODUCT OF STREPTOMYCIN INACTIVATION BY *E. COLI* CARRYING THE R-FACTOR

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Streptomycin was inactivated in the presence of ATP and magnesium ion by an enzyme preparation made from *E. coli* K 12 ML 1629 which is resistant to streptomycin. The structure of the inactivated streptomycin was determined, and it was found that the hydroxy group on C-3 of the N-methyl-L-glucosamine moiety of streptomycin is adenylylated.

Recently[1,2] it was found that in the presence of ATP and magnesium ion, kanamycin, paromamine and dihydrostreptomycin were inactivated by an enzyme preparation from *E. coli* K 12 ML 1629 carrying the R factor and which was resistant to these antibiotics. In another paper[1,3], it was reported that kanamycin and paromamine were inactivated by the phosphorylation at the hydroxy group on C-3 of the 6-amino-6-deoxy-D-glucose moiety or glucosamine moiety, and the inactivation of streptomycin was suggested also to be due to the phosphorylation. However, further studies, as briefly reported in a previous paper[4], indicated that the inactivated streptomycin is a compound consisting of adenylic acid and streptomycin. The structure of the inactivated streptomycin has now been determined to be adenylylstreptomycin in which the adenylic acid is combined with the hydroxy group on C-3 of N-methyl-L-glucosamine moiety. In this report, conditions for the inactivation of streptomycin by the enzyme prepared from *E. coli* K 12 ML 1629 and the isolation and structure of the inactivated streptomycin are reported.

Methods and Materials

(1) Organism:

In this investigation *E. coli* K12 CS2 and *E. coli* K12 ML1629 were employed. *E. coli* K12 CS2 is sensitive to chloramphenicol, tetracycline, kanamycin and streptomycin. *E. coli* K12 ML1629 was obtained from MITSUHASHI, Department of Bacteriology, School of Medicine, Gumma University. It was obtained by transmission of R-factor from a naturally isolated drug-resistant *E. coli* to *E. coli* K12 ML1410 which was resistant to nalidixic acid. *E. coli* K12 ML1629 is resistant to kanamycin, paromamine, destomycin, chloramphenicol, tetracycline and dihydrostreptomycin.

(2) Preparation of the Inactivating Enzyme Solution:

The test strain of *E. coli* was cultured in 100 ml of peptone broth (glucose 1 g, peptone 10 g, NaCl 3 g, 0.01 M $CaCl_2$ 10 ml, 0.1 M $MgCl_2$ 10 ml, 0.1 M KH_2PO_4 3.2 ml and 1 M NaOH 2.5 ml in 1 liter of distilled water) by shaking at 27°C for 20 hours. The cultured broth was then transferred to 900 ml of the same medium. When *E. coli* K12 ML1629 was

cultured, streptomycin was added to the above medium at 1 mcg/ml. The culture was incubated under aeration at 27°C for 7 hours and the broth was then chilled rapidly by pouring on crushed ice. The cells were collected by centrifugation (6,000×*g*, 20 minutes). The sedimented cells were washed twice with 1 liter of modified TMK solution (0.06 M KCl, 0.01 M $MgCl_2$ and 0.006 M 2-mercaptoethanol in 0.1 M tris buffer, pH 7.9) by centrifugation. The cells were suspended in modified TMK solution in a volume equal to the cell pellet and disrupted by passage through a French pressure cell (1,500 kg/cm²). The suspension of the disrupted cells was centrifuged at 30,000×*g* for 20 minutes and the supernatant was centrifuged at 100,000×*g* for 90 minutes. The supernatant thus obtained was treated with 2 mcg/ml of deoxyribonuclease (Worthington Biochemical Corporation) and 2 mcg/ml of ribonuclease (Worthington Biochemical Corporation) at 37°C for 15 minutes. It was then dialyzed against modified TMK solution. The enzyme solution thus prepared was diluted with modified TMK solution to contain 10 mg of protein per ml as determined by the FOLIN method.

(3) Inactivating Reaction of Streptomycin:

The reaction mixture consisted of the following materials: 0.3 ml of 10 times concentrated modified TMK solution (pH 8.3), 32 μmoles of disodium ATP which was neutralized with $NaHCO_3$, 8 μmoles of streptomycin sulfate and 0.3 ml of the enzyme solution in the total volume adjusted to 1.0 ml with distilled water. In some experiments, the amount of materials was changed as indicated. The reaction was carried out at 37°C for 20 hours. It was stopped by heating at 90°C for 5 minutes and the residual streptomycin was determined by the cylinder plate method using *B. subtilis* as a test organism.

(4) Reactivation of Inactivated Streptomycin with Phosphodiesterase and Alkaline Phosphatase:

The following reaction mixture was used: 100 mcg of inactivated streptomycin, 20 mcg of phosphodiesterase (Venom phosphodiesterase, Worthington Biochemical Corporation) or alkaline phosphatase (Alkaline phosphatase of calf mucosa type I, Sigma Chemical Company) and 0.5 ml of BMK solution (0.05 M KCl and 0.01 M $MgCl_2$ in 0.05 M borate buffer, pH 10.0) in a total volume adjusted with distilled water to 1.0 ml. The reaction was performed at 37°C for 5 and 10 hours.

(5) Periodate Oxidation[5] of Streptomycin, Inactivated Streptomycin and their Methanolysis Products:

The periodate oxidation was carried at 4°C for 24 hours in the following reaction mixture: 100 μmoles of compound and 1,000 μmoles of sodium periodate in 50 ml of 0.2 M acetate buffer (pH 4.1). The periodate consumption was determined by the FLEURY-LANGE method. One ml of ethyleneglycol was added to the reaction mixture and then hydrolyzed with 6 N HCl under reflux for an hour. The hydrolyzate was concentrated to a syrup *in vacuo* and washed with acetone. The residue was extracted with ethanol. The ethanol extract was studied by paperchromatography and high voltage paper electrophoresis.

(6) Preparation of N-Methyl-L-glucosamine HCl by Hydrolysis of Dihydrostreptomycin:

A solution of 30 g of dihydrostreptomycin sulfate in 200 ml of 1 N sulfuric acid was allowed to stand for 50 hours at 50°C. The solution was neutralized with sodium hydroxide and concentrated to a syrup *in vacuo*. The material giving a positive ELSON-MORGAN reaction was extracted with 200 ml of methanol, the extract was concentrated *in vacuo* and the residue was dissolved in 1 liter of water. The solution was passed through a 500 ml column of Dowex 50W-X4(OH). After washing, the ELSON-MORGAN positive fraction was eluted with 0.3 N HCl. This fraction was neutralized with Dowex 44(OH), decolorized with active carbon and concentrated *in vacuo* to an amorphous hygroscopic powder, 2.8 g. Crystals were obtained from an ethanol solution of the powder: m.p. 160.5~164°C (dec.); $[\alpha]_D^{20}$ −99.5° → −88.9° in 20 hours (c, 2.36 in water). Anal. calcd. for $C_7H_{15}NO_5 \cdot HCl$: C 36.61, H 7.02, N 6.10, O 34.83, Cl 15.44; found: C 35.73, H 7.52, N 5.68, O 34.53, Cl 14.47. The N.M.R. spectrum of its solution in deuterium oxide taken

at 60 Mc by the A-60 Varian Spectrometer showed signals of $>N-CH_3$ (+1.85 ppm from H-O-D, singlet) and an anomeric proton on C-1 (−0.89 ppm from H-O-D, doublet). Using the method of KUEHL *et al.*[6], acetylation of this amorphous powder gave pentaacetyl N-methyl-L-glucosamine, m. p. 159.5~160.5°C. Anal. calcd. for $C_{17}H_{25}NO_{10}$: C 50.61, H 6.25, N 3.47, O 39.66, CH_3CO 53.3; found: C 50.08, H 6.15, N 3.74, O 38.56, CH_3CO 51.08. From these results, the crystalline material was identified as N-methyl-α-L-glucosamine HCl.

(7) Preparation of Methyl Streptobiosaminide Dimethyl Acetal HCl:

A solution of 1.5 g of streptomycin hydrochloride in 100 ml of anhydrous methanol containing 1.7 % of hydrogen chloride was allowed to stand for 24 hours at 30°C. The solvent was neutralized with Dowex 44(OH) and concentrated *in vacuo*. The residue was dissolved in water and passed through a 20 ml column of IRC-50(Na). The ELSON-MORGAN positive fraction passed through and the effluent was concentrated to dryness *in vacuo*. The residue was extracted with ethanol - ether (2 : 1) and the solvent was removed *in vacuo* to give 850 mg of crude material. The crude material was dissolved in 5 ml of methanol - chloroform (1 : 1) and put on a 30 g column of silicic acid. The ELSON-MORGAN positive fraction was eluted with methanol - chloroform (1 : 3) and concentrated *in vacuo* to a light tan powder, 415 mg, $[\alpha]_D^{20}$ −138° (c, 1.25 in methanol). Anal. calcd. for $C_{13}H_{22}\cdot NO_7(OCH_3)_3\cdot HCl\cdot H_2O$: C 42.52, H 7.58, N 3.10, O 38.95, Cl 7.85; found: C 41.50, H 7.39, N 3.14, O 38.03, Cl 8.71.

(8) Preparation of AMP-Methyl Streptobiosaminide Dimethyl Acetal:

A solution of 320 mg of the inactivated streptomycin hydrochloride in 150 ml of anhydrous methanol containing 1.7 % of hydrogen chloride was kept at 30°C for 24 hours. The solvent was neutralized with Dowex 44(OH) and concentrated *in vacuo*. The residue was dissolved in 30 ml of water and put on a 20 ml column of IRC-50(Na). The ELSON-MORGAN positive fraction which passed the column was concentrated *in vacuo*. The residue was extracted with 4 ml of methanol - chloroform (1 : 1). The extract was passed through a 10 g column of silicic acid and eluted with methanol - chloroform (1 : 1). The eluate was concentrated *in vacuo* to 84 mg of a light tan powder, $[\alpha]_D^{20}$ −117.3° (c, 0.8 in methanol); $E_{1\%}^{1cm}$ 175 at 260 mμ. Anal. calcd. for $C_{13}H_{21}NO_7(OCH_3)_3C_{10}H_{13}O_6N_5PNa\cdot 3H_2O$: C 38.86, H 6.15, N 10.46, O 37.83; found: C 37.99, H 6.08, N 9.68, O 37.34. The N. M. R. spectrum of this matrial in deuterium oxide taken at 100 Mc by a JMN 4H-100 spectrometer (Japan Optics Laboratory) showed signals of $>N-CH_3$ (+1.93 ppm from H-O-D, singlet), an anomeric proton on C-1 of ribose (−1.30 ppm from H-O-D, doublet) and two aromatic protons on adenine (−3.32 ppm, singlet, and −3.55 ppm, singlet from H-O-D). From these results, it was identified to be AMP-methyl streptobiosaminide dimethyl acetal.

Results and Discussion

1. Conditions for Inactivation of Streptomycin

As shown in Table 1, the enzyme solution prepared from *E. coli* K 12 ML 1629 almost completely inactivates streptomycin after a reaction of 20 hours, whereas the supernatant of disrupted cells centrifuged at 100,000×*g* of *E. coli* K 12 CS 2 which is sensitive to streptomycin has no ability to inactivate streptomycin. When the enzyme solution prepared from the strain ML 1629 was heated at 90°C for 5 minutes, the ability to inactivate streptomycin was lost. Conditions for the inactivation of streptomycin were tested by the enzyme solution prepared from *E. coli* K 12 ML 1629.

The optimum pH of the inactivating reaction is 8.3~8.5, as shown in Fig. 1. In this experiment, the following buffers were employed: Tris-maleate buffer (pH 5.0~8.2), Tris-HCl buffer (pH 7.2~9.0) and glycine-NaOH buffer (pH 8.5~10.0) and the

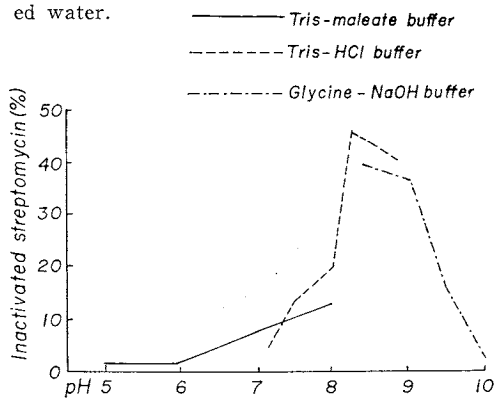
Table 1. Inactivation of streptomycin (SM) by enzyme solutions prepared from *E. coli* K12 CS2 and *E. coli* K12 ML1629

pH	strain	Residual SM (μg/ml) 0 hr.	Residual SM (μg/ml) 20 hr.	Inactivated SM (%)
7.9	CS-2	410	410	0
	CS-2	4,000	4,000	0
	ML-1629	400	0	100
	ML-1629	3,800	500	87
	ML-1629(heated)*	420	430	0
	ML-1629(heated)*	4,000	4,000	0
8.3	CS-2	400	400	0
	CS-2	4,000	4,000	0
	ML-1629	390	0	100
	ML-1629	4,000	100	97
	ML-1629(heated)*	445	450	0
	ML-1629(heated)*	4,000	4,000	0

* Enzyme from *E. coli* K12 ML1629 was inactivated by heating at 90°C for 5 minutes.

Fig. 1. Effect of pH on inactivation of streptomycin

Reaction mixture contained the following materials : 100 μg of streptomycin sulfate, 480 μg of ATP, 0.6 ml of 0.1 M buffer. 0.03 ml of enzyme solution and 0.1 ml of 0.1 M $MgCl_2$ in a total volume adjusted to 1.0 ml with distilled water.



ratio of inactivated streptomycin to streptomycin added to the reaction mixture at each pH is shown as per cent. Three mg of enzyme as protein per ml of the reaction mixture inactivated more than 95 % of 8 μmoles of streptomycin in the presence of 4 times moles of ATP at optimum pH, and inactivated less than 55 % of the streptomycin when more than 10 μmoles of streptomycin was added to the reaction mixture. When equimolar amounts or two-times this quantity of ATP was added as compared to streptomycin, less than 50 % of the added streptomycin was inactivated.

It was known that the phosphorylative inactivation of kanamycin required only ATP as a phosphate donor. The requirement of nucleotide for the inactivation of streptomycin in the above system was tested. As shown in Table 2, streptomycin was not inactivated without nucleotide. When ATP and ADP was added to the reaction mixture, 96.9 % and 59.0 % of the streptomycin was inactivated respectively at the optimum pH. ATP and ADP could not be replaced by AMP, GTP, UTP and CTP. In the reaction mixture containing 8 μmoles of streptomycin sulfate, 32 μmoles of ADP and 0.3 ml of the enzyme solution in 1 ml of the reaction mixture, the decrease of ADP and the production of ATP and AMP were confirmed. At 1 hour after the reaction, 5.2 μmoles per ml of ATP was produced maximally. In this experiment, the reaction was stopped by

Table 2. Effect of nucleotides on inactivation of streptomycin (SM)

pH	Nucleotide	Residual SM (μg/ml) 0 hr.	Residual SM (μg/ml) 20 hr.	Inactivated SM (%)
7.9	none	400	380	5.0
	ATP	457	35	92.1
	ADP	430	171	60.2
	AMP	455	445	2.2
	GTP	470	470	0
	UTP	491	499	0
	CTP	430	450	0
8.3	none	410	380	7.3
	ATP	451	18	96.9
	ADP	450	266	59.0
	AMP	450	420	6.7
	GTP	430	439	0
	UTP	441	460	0
	CTP	400	400	0

Reaciton mixture contained the following materials : 0.8 μmole of streptomycin sulfate, 3.2 μmole of nucleotide, 0.3 ml of 10 times concentrated modified TMK solution and 0.1 ml of enzyme solution in a total volume adjusted to 1.0 ml with distilled water.

addition of an equal volume of 16 % of trichloroacetic acid and ATP, ADP and AMP in the supernatant was determined by the paper chromatography using a solvent system of iso-butylic acid - acetic acid - 1 N NH_4OH (10 : 1 : 5). Using this procedure, when the enzyme solution was omitted, the ADP was not changed. It is believed that the enzyme solution used, converted the ADP to ATP which was then used for the inactivation reaction.

Magnesium ion was found to be necessary for inactivation of streptomycin. When 0.5 μmole of streptomycin, 2 μmoles of ATP and 0.3 mg of the enzyme as protein were added to 1 ml of the reaction mixture, with addition of 5.3~10.3 μmoles of $MgCl_2$ per ml of the reaction mixture, more than 90 % of the streptomycin was inactivated for 24 hours, but addition of 0.3 μmole of $MgCl_2$ per ml caused the inactivation of only 6 % of the streptomycin. When 5 μmoles of streptomycin, 20 μmoles of ATP and 3 mg of the enzyme as protein were added to 1 ml of the reaction mixture, streptomycin was almost completely inactivated with the addition of 13~63 μmoles of $MgCl_2$ per ml of the reaction mixture and addition of $MgCl_2$ at 3 μmoles per ml caused the inactivation of 45 % of the streptomycin.

Thus it was concluded that the enzyme solution obtained from *E. coli* K12 ML1629 required ATP and magnesium ion for the inactivation of streptomycin.

2. Isolation of Inactivated Streptomycin

In order to isolate the inactivated streptomycin, a large amount of streptomycin was inactivated by the enzyme solution prepared from *E. coli* K 12 ML 1629. A reaction mixture which contained 560 mg of streptomycin sulfate, 3,600 mg of disodium ATP, 980 mg of $NaHCO_3$, 40 ml of 10 times concentrated modified TMK solution and 30 ml of the enzyme solution was adjusted to 100 ml with distilled water. After reaction at 37°C for 20 hours, 80 % of the streptomycin was inactivated.

The inactivated streptomycin solution was passed through a 200 ml column of Amberlite IRC-50(Na) and eluted with 0.5 N HCl. The fraction which gave a positive SAKAGUCHI reaction and which showed no antibacterial activity was collected and put on a 20 g column of active carbon. The inactivated streptomycin was eluted with 0.1 N HCl in 50 % aqueous methanol. The fraction giving a positive SAKAGUCHI reaction and negative biological activity was cut and neutralized with Dowex 44(OH). After concentration under vacuum, the inactivated streptomycin was precipitated by addition of 14 volumes of acetone and dried *in vacuo*. A part of the inactivated streptomycin in the reaction mixture passed the column of IRC-50 resin, but it was adsorbed by another column of the same resin and purified by the same procedure as described above. Totally, 250 mg of inactivated streptomycin were obtained.

3. Characteristics of Inactivated Streptomycin

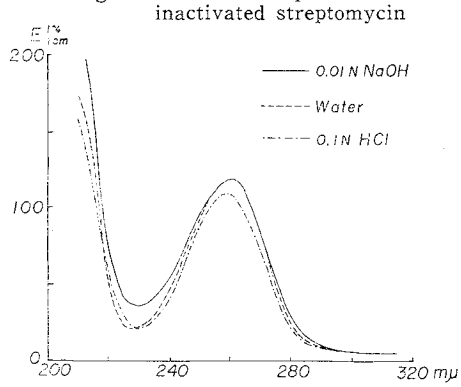
The inactivated streptomycin thus obtained was positive for SAKAGUCHI reaction, ELSON-MORGAN reaction, maltol reaction and also HANES' reagent for phosphorus. The inactivated streptomycin showed one spot in the following paper chromatographic systems: Rf 0.18 with water adjusted to pH 10.0 with aqueous ammonia, Rf 0.08 with methanol - ethanol - conc. HCl - water (50 : 25 : 6 : 19), Rf 0 with *n*-butanol saturated with water - 28 % aqueous ammonia (100 : 1) and Rf 0 with *n*-butanol - acetic acid -

Table 3. Reactivation of inactivated streptomycin by alkaline phosphatase and phosphodiesterase

Phosphatase	Recovered activity (μg/ml)		
	0 hr.	5 hr.	10 hr.
None	0	0	0
Alkaline phosphatase	0	36.1	43.2
Phosphodiesterase	0	31.4	36.9

Reaction mixture contained the following materials : 100 μg of inactivated streptomycin, 20 μg of phosphatase and 0.5 ml of BMK solution in a total volume adjusted to 1.0 ml with distilled water. The reaction was made at 37°C.

Fig. 2. Ultraviolet spectrum of inactivated streptomycin



water (4 : 1 : 1). Rf values of streptomycin with these solvents were 0.12, 0.20, 0 and 0.02 respectively. In high voltage paper electrophoresis using acetic acid - formic acid - water (75 : 25 : 900) under 3,500 volts for 15 minutes, the inactivated streptomycin moved 10.5 cm towards the cathode and streptomycin 13.0 cm. As shown by the electrophoresis study, the inactivated streptomycin was less basic than streptomycin. The inactivated streptomycin showed a maximum at 260 mμ in the ultraviolet spectrum as seen in Fig. 2, when it was dissolved in distilled water, 0.1 N HCl and 0.01 N NaOH and the absorption spectrum was similar to that of adenylic acid except for the optical density. Determination of streptomycin by the maltol reaction, determination of adenosine by optical density at 260 mμ and determination of phosphorus by ALLEN's method indicated the presence of streptomycin, adenosine and phosphorus in the molar ratio of 1 : 1 : 1 in the inactivated streptomycin.

Anal. calcd. for $C_{21}H_{38}O_{12}N_7 \cdot C_{10}H_{12}O_6N_5PNa \cdot 2HCl \cdot 4H_2O$:
C 34.55, H 5.61, O 32.66, N 15.60, P 2.87, Cl 6.58, Na 2.13.
Found : C 34.65, H 6.02, O 29.97, N 13.39, P 2.50, Cl 6.04, Na 1.36.

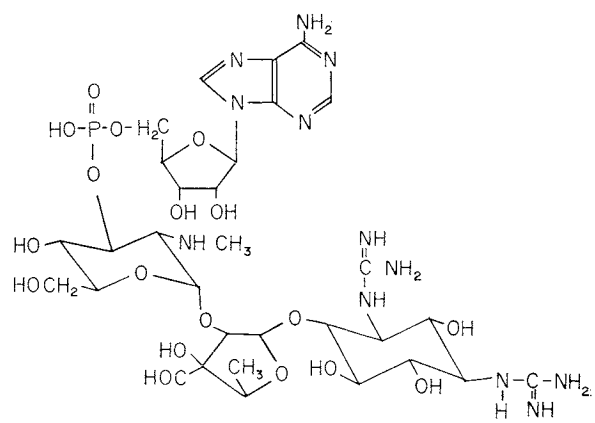
The reactivation of the inactivated streptomycin with phosphatase was tested. As shown in Table 3, the inactivated streptomycin was reactivated by both alkaline phosphatase and phosphodiesterase and 432 mcg of streptomycin per mg was recovered after 10-hour treatment. Thus 80 % of the inactivated streptomycin was reactivated. The activities of the alkaline phosphatase and phosphodiesterase used were tested by determination of *p*-nitrophenol after hydrolysis of *p*-nitrophenyl phosphate and bis-*p*-nitrophenyl phosphate as described by OHSAKA *et al.*[7]. Although the alkaline phosphatase employed hydrolyzed both *p*-nitrophenyl phosphate and bis-*p*-nitrophenyl phosphate in BMK solution at 37°C for 5 hours, phosphodiesterase hydrolyzed the former but not the latter even after 10 hours. From these results, it was considered that a considerable amount of diesterase was contained in the alkaline phosphatase. In the reaction of the alkaline phosphatase, the inactivated streptomycin was hydrolyzed to streptomycin and adenosine by action of both the phosphodiesterase and alkaline phosphatase.

Hydrolyzates of the inactivated streptomycin obtained with hydrochloric acid, alkaline phosphatase and phosphodiesterase were tested by paper chromatography in the following solvent systems : water adjusted to pH 10.0 with aqueous ammonia,

methanol – ethanol – conc. HCl – water (50 : 25 : 6 : 19), *n*-butanol – acetic acid – water (4 : 1 : 1) and isobutyric acid – acetic acid – 1 N NH_4OH (10 : 1 : 5). The acid hydrolysis of the inactivated streptomycin was done using 1 N HCl in boiling water for one hour. On the paper chromatograms, adenine (Rf values were 0.41, 0.38, 0.34 and 0.95 respectively) was detected in the acid hydrolyzate. Adenosine (Rf values ; 0.54, 0.37, 0.48, 0.88) and streptomycin (Rf values ; 0.12, 0.20, 0.02, –) were detected in the hydrolyzate of alkaline phosphatase, and AMP (Rf values ; 0.95, 0.50, –, 0.69) and streptomycin in the phosphodiesterase hydrolyzate. However, ATP, ADP, guanosine, inosine, thymidine, uridine, cytidine, guanine and hypoxanthine were not detected in any hydrolyzates.

The consumption of periodate by inactivated streptomycin, streptomycin, AMP-methyl streptobiosaminide dimethyl acetal and methyl streptobiosaminide dimethyl acetal were tested. The inactivated streptomycin, AMP-methyl streptobiosaminide dimethyl acetal and methyl streptobiosaminide dimethyl acetal consumed 2.8, 1.2 and 2.2 moles of periodate respectively at pH 4.1 for 24 hours while streptomycin consumed 4.0 moles. The acid hydrolyzates of the periodate oxidation products of the inactivated streptomycin and AMP-methyl streptobiosaminide dimethyl acetal gave the positive ELSON-MORGAN reaction but streptomycin and methyl streptobiosaminide dimethyl acetal did not. The ethanol extraction of the acid hydrolyzate of periodate oxidation product was tested by paper chromatography and high voltage paper electrophoresis. The ELSON-MORGAN positive material from the inactivated streptomycin and AMP-methyl streptobiosaminide dimethyl acetal was identified to be N-methyl glucosamine by the paper chromatograms using the following solvent systems : propanol – pyridine – acetic acid – water (51 : 20 : 6 : 24), *n*-butanol – acetic acid – water (4 : 1 : 5), *n*-butanol – ethanol – water (4 : 1 : 5), *n*-butanol – ethanol – water – 28 % ammonia (40 : 10 : 49 : 1) and methanol – ethanol – conc. HCl – water (50 : 25 : 6 : 19) and Rf values were 0.50, 0.17, 0.14, 0.26 and 0.54 respectively. Moreover, it was also identified to be N-methyl glucosamine by high voltage paper electrophoresis which moved to the cathode by 11.5 cm at 3,500 volts for 20 minutes.

The structure shown below can be assigned by the following basis : the inactivated streptomycin was reactivated by phosphodiesterase, streptomycin and adenylic acid were detected in the phosphodiesterase hydrolyzate, the molar ratio of streptomycin, adenosine and phosphorus in the inactivated streptomycin was 1 : 1 : 1, 3 moles of periodate was consumed by the inactivated streptomycin and 1 mole of periodate was consumed by the AMP-methyl streptobiosaminide dimethyl acetal, and N-methyl glucosamine remained in the acid hydrolyzate of the periodate oxidation product of the inactivated streptomycin and AMP-methyl streptobiosaminide dimethyl acetal.



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