Abstract

This paper presents the effects of extracellular ATP, ADP, AMP, and adenosine on the occurrence of streptomycin resistance in E.coli.

Results from this research show that extracellular ATP and adenosine decreased the occurrence of streptomycin resistant mutants, while extracellular ADP and AMP increased the occurrence of streptomycin resistant mutants.

These compounds appear to influence the mutatability of E.coli for resistance to streptomycin by affecting the levels of Mg^{2+} in the cell.

Suppression of Drug Resistance in E.coli by Reduction in Mg^{2+} using Adenosine

A small innocula of wild type E.coli was isolated from fecal matter and grown in a nutrient beef broth culture for 24 hours. Slants containing 0.5ml of media were cultured with innocula from the broth culture. Each set of slants was cultured synchronously (Table 1: each row represents a set of slants). After an incubation period of 8 hours, 10µl of each slant was cultured on individual nutrient agar plates. A colony count was taken after 24 hours.

Overall, cultures that included ATP and adenosine in addition to streptomycin showed a marked drop in the number of mutant colonies compared to streptomycin only. Cultures that included ADP and AMP in addition to streptomycin showed a significant increase in the number of mutant colonies.

Streptomycin acts by binding to the 30S ribosome, preventing the initiation of protein synthesis[(1)]. Since the presence of Mg^{2+} enables fMet tRNA to bind to the 30S unit even in the presence of streptomycin[(1)] the experiment was repeated with five types of media (Data not shown), which included; (1) streptomycin, (2) streptomycin with adenosine, (3) streptomycin with adenosine and magnesium (in the form of magnesium sulphate), (4) streptomycin with magnesium, and (5) adenosine .

The cultures, in which magnesium was included, showed jackpot number of colonies (non-confluent), while the cultures treated with streptomycin only and streptomycin with adenosine displayed high level of mutation suppression. The cultures with only adenosine showed normal confluent growth.

The inability of the cells to mutate in response to streptomycin when only adenosine is added (Table 1: Columns 9, 10), and the ability to mutate when magnesium is added to the streptomycin and adenosine indicates that adenosine suppresses mutation in the first instance (Sm+ADO), by binding with the free Mg^{2+}. When the amount of free Mg^{2+} in the second instance (Sm+ADO+Mg^{2+}) exceeds its uptake by adenosine, the excess Mg^{2+} probably enables increase in the occurrence of mutation in the presence of streptomycin.

Cultures treated with ATP appear to have an effect of suppressing mutation. Addition of extracellular ATP that is not bound to Mg^{2+}probably decreases the level of free Mg^{2+} (which would be otherwise available on the hydrolysis of $MgATP^{2-}$ available in the cell [(2)]), due to ATP being hydrolysed instead of $MgATP^{2-}$.

However the occurrence of a confluent growth (Table 1: L3), and increased number of mutant colonies (Table 1: A3, A4, I3, N4) may be due to cellular $MgATP^{2-}$ being hydrolysed (due to cell division taking place) prior to the addition of extracellular ATP, which results in free Mg^{2+} being available and thereby results in a high number of mutant cells.

Cultures treated with ADP have higher instances of mutant colonies (13 out of 30 cultures) indicating that the presence of ADP may generally increase mutation to streptomycin. This may be caused by the cell's response of increasing $MgATP^{2-}$ synthesis in response to $MgATP^{2-}$/ADP imbalance caused by the addition of extracellular ADP[(3)].

In the case of AMP, the cell's capability to mutate increased significantly. Addition of extracellular AMP may have increased the level of Mg^{2+} due to increase in the level of $MgATP^{2-}$ and its subsequent hydrolysis caused by the added AMP[(3)].

Adenosine significantly reduced the cell's capability to mutate. Compared to ATP, which sometimes displayed inability to suppress mutation (Table 1: A3, A4, I3, N4), adenosine consistently displayed an ability to suppress mutation. Adenosine binds to the free Mg^{2+}resulting in decrease of free Mg^{2+} as we have seen earlier.

In summation, the availability of Mg^{2+} due to $MgATP^{2-}$ hydrolysis prior to cell division, or by the addition of extracellular ADP and AMP, may cause structural modification of the 30S ribosome of streptomycin sensitive cells, which are treated with streptomycin. This enables fMet tRNA to bind to the 30S subunit, an action that is normally blocked by streptomycin binding to the 30S subunit's active site to prevent the initiation of protein synthesis [(4)].

Therefore, lower levels of Mg^{2+} caused by addition of ATP or adenosine may result in inability of fMet tRNA to bind to the 30S subunit due to the action of streptomycin. This results in the prevention of protein synthesis initiation and cell death.

The ability of adenosine to reduce mutatability may be researched further with other pathogens for suppression of antibiotic resistance. ATP may not be as effective since this research indicates that availability of excess $MgATP^{2+}$ that may occur prior to cell division may still enable mutation.

If adenosine is found to be effective, it can be co-administered or may be made an additional ingredient, where it is mechanically bonded to the compound. Adenosine is being manufactured and sold commercially for the treatment of supraventricular tachycardia, and much is known about its contraindications[(5)]. Adenosine may be used along with an agonist to prevent drug-resistance.

Alternatively, other methods of reducing magnesium levels during drug administration may also be attempted.

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Table 1

	*Streptomycin only (2mg/1000ml)		ATP (1mg/100ml of Sm solution with conc. of 2mg/1000ml)		ADP (1mg/100ml of Sm solution with conc. of 2mg/1000ml)		AMP (1mg/100ml of Sm solution with conc. of 2mg/1000ml)		ADO (1mg/100ml of Sm solution with conc. of 2mg/1000ml)	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
A	127	286	19	15	17	4	92	62	6	6
B	2	1	0	8	1	5	4	4	0	0
C	0	0	0	0	3	48	58	4	3	2
D	2	2	2	0	14	2	64	54	0	1
E	1	1	0	2	1	1	2	8	1	2
F	0	1	0	0	0	2	Con	1	2	0
G	2	1	0	3	1	1	65	45	13	2
H	0	0	1	0	1	26	63	63	1	0
I	2	0	10	6	54	39	51	36	0	1
J	0	1	2	1	22	1	41	37	1	0
K	1	1	6	0	2	41	148	93	6	0
L	1	0	Con	6	6	27	67	176	0	0
M	1	1	3	3	2	29	121	183	0	4
N	0	1	0	23	46	0	32	123	0	0
O	8	5	4	1	138	19	92	28	0	6
Avg. mean for series	9.8	20.07	29.8	4.5	20.5	16.3	86.7	61.13	2.2	1.6
Avg. mean for media		14.93		17.17		18.43		73.9		1.9