SC 2187

Biosynthesis of 7-chloro-6-demethyltetracycline in the presence of aminopterin and ethionine

It has been demonstrated that [*Me*-^{14}C]methionine and [2-^{14}C]-glycine are efficiently incorporated into 7-chlorotetracycline[1], and that the three methyl groups of 5-hydroxytetracycline are derived from methionine[2,3]. These findings have led several investigators to study the effects on tetracycline biosynthesis of various antimetabolites of compounds functioning in biological methylation. It has been shown that sulfa drugs[4,5] and ethionine[6] cause the production of 7-chloro-6-demethyltetracycline by strains which do not normally synthesize this substance. A methionine-requiring strain of *Streptomyces viridifaciens*, producing 7-chloro-6-demethyltetracycline, gave increased yields of this antibiotic with ethionine[6].

TABLE I

7-CHLORO-6-DEMETHYLTETRACYCLINE PRODUCTION WITH AMINOPTERIN AND VARIOUS COMPOUNDS

Inhibitor	*Concn. (mM)*	*Reversing compound*	*Concn. (mM)*	*Diameter of inhibition zone on bioautogram (mm)*		
				*CTC**	*DCTC**	*TC**
Aminopterin	1.1	None	0	32	27	21
Aminopterin	1.1	L-Methionine	6.7	30	tr*	16
Aminopterin	1.1	α-methyl-DL-Methionine	6.2	33	tr	21
Aminopterin	1.1	DL-Homocysteine	5.6	28	15	tr
Aminopterin	1.1	Cyanocobalamin	0.37	30	22	18
Aminopterin	1.1	*p*-Aminobenzoic acid	7.4	30	20	20
Aminopterin	1.1	Folic acid	2.2	32	22	18
None	0	None	0	32	0	30

* tr = trace; CTC = 7-chlorotetracycline; DCTC = 7-chloro-6-demethyltetracycline; TC = tetracycline.

TABLE II

EFFECT OF AMINOPTERIN AND VARIOUS COMPOUNDS ON TETRACYCLINE POTENCY[4]

Compound added	*Concn. (mM)*	*Tetracycline potency, (μg/ml)*	
		*+ aminopterin**	*— aminopterin*
None	0	854	1230
L-Methionine	6.7	517	412
α-methyl-DL-Methionine	6.2	1100	1210
DL-Homocysteine	5.6	290	805
Folic acid	2.2	840	829

* Final concentration aminopterin, 1.1 mM.

We have extended these studies with ethionine and, further, we have shown that *Streptomyces aureofaciens* ATCC 13900 which produces 7-chlorotetracycline and tetracycline, but not 7-chloro-6-demethyltetracycline, will synthesize this latter substance when grown in the presence of aminopterin.

The organism was grown in autoclaved medium containing extraction process soybean meal, glucose, NaCl, and powdered $CaCO_3$ with sterile additives. After a

TABLE III

7-CHLORO-6-DEMETHYLTETRACYCLINE PRODUCTION WITH ETHIONINE AND VARIOUS COMPOUNDS

Inhibitor	*Concn. (mM)*	*Reversing compound*	*Concn. (mM)*	*Diameter of inhibition zone on bioautogram (mm)*		
				*CTC**	*DCTC**	*TC**
L-Ethionine	0.12	None	0	30	20	tr
L-Ethionine	0.31	None	0	10	0	tr
L-Ethionine	0.62	None	0	0	0	0
L-Ethionine	0.62	L-Methionine	20.1	28	17	tr
L-Ethionine	0.62	L-Methionine	3.3	27	0	0
L-Ethionine	0.62	α-methyl-DL-Methionine	6.2	tr	0	tr
L-Ethionine	0.62	DL-Methionine sulfoxide	6.1	26	0	15
L-Ethionine	0.62	DL-Methoxinine	0.75	35	23	14
L-Ethionine	0.31	Glycine	13.3	25	0	tr
L-Ethionine	0.31	DL-Serine	9.5	22	0	tr
L-Ethionine	0.31	DL-Threonine	8.4	30	0	tr
D-Ethionine	0.62	None	0	24	20	10
DL-Ethionine	0.62	None	0	18	tr	tr
DL-Ethionine	0.62	DL-Homocysteine	2.8	25	tr	tr
DL-Ethionine	0.62	Cyanocobalamin	0.0074	30	0	17
DL-Ethionine	0.62	Co^{2+} (as $Co(NO_3)_2$)	0.17	25	17	18
None	0	None	0	34	0	22

* See Table I.

TABLE IV

EFFECT OF ETHIONINE AND VARIOUS COMPOUNDS ON TETRACYCLINE POTENCY

Compound added	*Concn. (mM)*	*Tetracycline potency (μg/ml)*	
		*+ ethionine**	*− ethionine*
None	0	302	1020
DL-Methionine	6.7	664	795
DL-Methoxinine	0.75	994	1150
DL-Methionine sulfoxide	6.1	620	1130
DL-Homocysteine	2.8	512	555
DL-Threonine	8.4	564	880
p-Aminobenzoic acid	0.74	356	926
Folic acid	0.22	338	880
Cyanocobalamin	0.0074	736	894
Co^{2+} (as $Co(NO_3)_2$)	0.017	750	750

* Final concentration DL-ethionine, 0.62 mM.

7-day incubation at 25° on a rotary shaker, samples were acidified to pH 2.5 with H_2SO_4 and analyzed for tetracycline antibiotics by filter-paper chromatography and bioautography using *Staphylococcus aureus* 209P (refs. 5, 7, 8). Semiquantitation was achieved by measurement of the diameters of the zones of inhibition in the bioautograms. In addition, quantitative data for the total tetracycline potency in the acidified fermentation samples were obtained using a method based on the inhibition of CO_2 evolution by *Escherichia coli*[9].

The data summarized in Tables I and II show that the production of 7-chloro-6-demethyltetracycline by *S. aureofaciens* ATCC 13900 grown in media containing aminopterin is reversible by addition of L-methionine and α-methyl-DL-methionine.

It is to be further noted that L-methionine alone and aminopterin only in combination with DL-homocysteine are inhibitory to tetracycline biosynthesis.

In Tables III and IV, it can be seen that L- and DL-ethionine are quite inhibitory to tetracycline synthesis, in part due to growth inhibition, but that these compounds as well as D-ethionine cause production of 7-chloro-6-demethyltetracycline. Certain chemical agents can increase or eliminate biosynthesis of this substance and overcome the general inhibition of tetracycline production in the presence of ethionine. These agents include methionine, methionine sulfoxide, methoxinine, glycine, serine, threonine, homocysteine, cyanocobalamin, and Co^{2+} ions.

The inhibition of the 6-methylation of 7-chlorotetracycline by aminopterin supports the hypothesis offered in an earlier report[4] that the insertion of this methyl group is a folic acid-dependent reaction. The effects of ethionine may be ascribed to either a direct competition with methionine or an ethylated folic acid derivative acting as an antagonist of the normal methylated coenzyme.

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Unavailability of chromaffin granule adenosine triphosphate for metabolic reactions

The adrenal medulla contains considerably more ATP than any other tissue thus far studied[1]. Almost all of this ATP is held within the chromaffin granules[2], which store the adrenaline and noradrenaline[3]. The adrenaline and noradrenaline are known to be held within the chromaffin granules in a biologically inactive form. Thus, when a preparation of isolated adrenal medulla "large granules" suspended in isotonic sucrose is injected intravenously into a cat only a small fraction of the pressor activity of the contained amines is immediately apparent. On the other hand, if the granules are first "lysed" in distilled water and then injected the full pressor activity of the contained amines is immediately observed[4].