INHIBITION OF BACTERIAL DNA METHYLATION BY S-TUBERCIDINYLHOMOCYSTEINE (STH)

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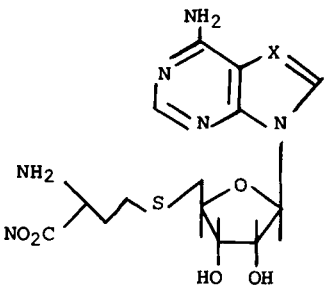

SAH X = N

STH X = CH

S-Tubercidinylhomocysteine (STH) (Coward et al, 1974) designed as a metabolically stable (Crooks et al, 1979) synthetic analogue of S-adenosylhomocysteine (SAH), the natural feed-back inhibitor of S-adenosylmethionine (SAM)-dependent methylases was prepared by the reaction of 5'-chlorotubercidin (Crooks et al, 1979) with homocysteine in Na-liquid NH_3. Purification of the product by preparative high-pressure liquid-chromatography on a Magnum-10 ODS column using $CH_3OH:H_2O$ (20 : 80 v/v) as mobile phase afforded STH of high analytical purity. We have previously reported the inhibition of t-RNA methylation by STH in cultured Novikoff hepatoma cells (Coward and Crooks, 1979).

Bacterial DNA methylation was assayed in extracts of *Escherichia coli* J6-2 *dcm*$^+$ (proficient in DNA cytosine methylase activity) disrupted by ultrasonic treatment. A reaction mixture consisting of 100µl of DNA extracted from *E. coli* J6-2 *dcm*$^-$ (lacking in DNA cytosine methylation), 200 µl of cell extract and 15 µl of S-adenosyl-L [methyl-^{3}H] methionine (specific activity 500 mCi/ m mole) diluted in water from 1mM to a suitable concentration was used. The assay was conducted at 37°. and started by the addition of the cell extract to the other two components. 50 µl samples were taken at suitable time intervals onto 2 cm^2 Whatman 3MM chromatography paper strips which were then immersed in ice cold 5% trichloroacetic acid. The samples were washed with 5% trichloroacetic acid solution, then with ethanol-ether solution, the papers dried and radioactivity estimated by scintillation spectrometry.

Concentrations of SAM used were 2.5µM, 3.3µM, 10µM and 20µM. Initial velocities of the reactions in the presence and absence of STH were obtained using the same freshly prepared cell extract. STH was tested at 20µM and gave aporoximately 70% inhibition of the reaction when SAM was at 2.5µM. The Km value obtained for SAM was 15µM, which agrees well with the value quoted by Razin et al (1975) using a similar bacterial system. Lineweaver-Burke plots of results obtained in the presence of STH showed that STH is a competitive inhibitor of bacterial DNA methylation and exhibited a Ki value of 18µM.

Thus both mammalian and bacterial nucleic acid methylation is inhibited by STH.

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