EFFECT OF CARBOHYDRATES ON INDUCTION OF BACTERIOPHAGE LAMBDA

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Summary: In our screening for antitumor antibiotics using induction of bacteriophage lambda as an in vitro marker, it was found that a monosaccharide induced the phage from lysogenic Escherichia coli. The phage inducing activity was investigated by an agar diffusion method for nine monosaccharides, five disaccharides, one trisaccharide and three related compounds. A most remarkable activity was shown by mannose, maltose, melibose, trehalose and glucuronolactone.

Certain antitumor agents, such as azaserine[1] and mitomycin[2], are known to be capable of inducing lambda bacteriophage in lysogenic Escherichia coli. This ability has been correlated with mutagenic and carcinogenic activities. Lein et al.[3] reported a correlation in the ability of antibiotics to induce lambda phage in lysogenic E. coli K-12 to inhibit transplanted tumors in animals. Others have also attempted to apply induction activity as a screen for potential antitumor agents[4,5].

In a search for antitumor agents with a lambda phage induction screen, the culture filtrate of Cercospora crusei, a plant pathogenic fungus, showed strong activity. Upon isolation the active principle was a colorless amorphous powder and was identified as a monosaccharide. This finding led us to examine several mono- and disaccharides and related compounds.

Methods

The agar diffusion method was used for the lambda induction as follows: E. coli K-12 grown for 18 hr at 38°C in a nutrient broth

was harvested by centrifugation and dilutedwith broth to 10^6 cells/ml. The indicator organism, E. coli W-3102, grown under the same conditions, was used without dilution. One hundred ml of the nutrient agar, melted and maintained at 45-48°C, was seeded with 1 ml of the diluted E. coli K-12 and 2 ml of indicator strain, thoroughly mixed and 10 ml poured into a 9 cm diameter petri dish. After the agar solidified, paper disks treated with the test samples were placed on the agar surface. Each sample to be tested was dissolved in distilled water at 10 and 100 mg/ml. A filter paper disk, 8 mm in diameter with an absorbing capacity of 50 µl was impregnated with each solution, the disk placed on the assay plate, and after incubation overnight at 37°C the diameter of the plaque forming zone measured in mm.

Results and Discussion

Lambda phage induction activity of the nine monosaccharides, five disaccharides, one trisaccharide and three related compounds tested is shown in Table 1. Most of the carbohydrates tested exerted some activity in this sytem.

Those which did not were sorbose, sucrose and raffinose. Some inhibition of the growth of the indicator strains without induction activity, was observed with galactose and lactose. The other compounds tested showed induction activity to some extent and most exerted growth stimulatory effect on the indicator as well. The methyl pentoses, fucose and rhamnose, showed significant induction activity but the growth stimulus was relatively weak. Glucurono-lactone was unique in its remarkable induction activity without other effect on the organism. Maltose, trehalose and malibiose were very active forming the largest induction zones.

Recently attention to the biological activities of carbohydrates includes, for example, a loss in transplantability by ascites tumor

cells on inhibition with a variety of monosaccharides, especially glucosamine and mannose[6]. Other examples are a stimulation in the transformation in Rous sarcoma virus infected chick embryo fibroblast[7] by DEAE-dextran and the activity of phytohemagglutinin on mammalian cells[8].

The present finding, and the evidence cited above, strongly suggest that carbohydrates themselves would affect the macromolecule

Table 1

Effect of carbohydrates on induction of bacteriophage lambda

Carbohydrates	Diameters of induction zones (mm)	
	0.5 mg/disk	5.0 mg/disk
Xylose	-	11.0
Arabinose	-	11.0
Galactose	-	-
Glucose	+	11.0
Mannose	+	19.0
Sorbose	-	-
Fructose	12.0	16.5
Rhamnose	12.0	14.5
Fucose	13.5	14.0
Sucrose	-	-
Lactose	-	-
Maltose	11.0	21.0
Trehalose	11.0	20.0
Melibiose	-	20.0
Raffinose	-	+
Glucosamine-HCl	+	13.0
Glucuronolactone	+	23.0
Glycerol	+	12.0

syntheses without further metabolism, since carbohydrates which are not utilized by E. coli are able to induce the lambda phage. These findings may in addition afford a clue to the mechanism of phage induction.

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