LOCALIZATION OF ISOTOPE FROM [2-^{14}C] GLUCOSE AND [6-^{14}C] GLUCOSE IN DEOXYPENTOSE MOIETIES OF HERPES SIMPLEX DNA

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SUMMARY: Deoxypentose of herpes simplex DNA is heavily labeled when viral infected tissue cultures are grown in the presence of [2-^{14}C] glucose and [6-^{14}C] glucose, but not in the presence of [1-^{14}C] glucose. Very little isotope is found in purine and pyrimidine bases of herpes DNA. Data indicate that glucose provides pentose components as well as an energy source for herpes virus synthesis.

Energy yielding systems of the cell such as glycolysis and the shunt are enhanced when herpes simplex virus (HF strain) multiplies in tissue culture (1). Recent experiments indicate that herpes DNA incorporates large amounts of isotope when host-virus preparations are grown in the presence of [2-^{14}C] glucose or [6-^{14}C] glucose, but very little isotope is incorporated when [1-^{14}C] glucose is added to the medium (2). Experiments designed to isolate radioactive deoxypentose components from herpes simplex DNA are reported below. These experiments indicate that most of the radioactivity resides in viral deoxypentose components, while very little isotope is found in purine and pyrimidine bases of herpes DNA.

MATERIALS AND METHODS

Four day old H. Ep. 2 cell cultures, grown on glass (1) were maintained 24 hr in medium without glucose in order to minimize existing internal cellular pools of the compound, thereby insuring isotope assimilation. Cell cultures were treated with Kantrex (100 µg/ml medium) to exclude the possibility of interference by PPLO agents. Virus (2) and isotope were introduced

into each of 40 H. Ep. 2 cell bottle cultures and virus was allowed to adsorb 2 hr at room temperature (22°). Cultures were moved to 35° for 2 hr, after which time enough sterile cold glucose was added to each viral infected system in order to provide a final concentration of 0.05%. Cultures were incubated 26 hr at 35°. Infected cells were harvested in dilute saline-citrate buffer (3) containing 0.01 M EDTA. Cells were broken by freezing and thawing 3 times; cellular debris was sedimented at 1500 xg while virus was recovered by centrifuging these supernatant fluids at 66,000 xg in the model L2-65 ultracentrifuge. Viral pellets were resuspended and treated with DNase (50 μg/ml)

TABLE I

ISOTOPE INCORPORATION FROM RADIOACTIVE GLUCOSE

INTO DEOXYRIBOSE OF HERPES SIMPLEX DNA

Herpes infected H. Ep. 2 cells, in 26 hr growth experiments, were cultivated at 35° under stationary conditions in the presence of isotope using 30-70 μc [2-^{14}C] glucose, 50 μc [6-^{14}C] glucose, and 90 μc [1-^{14}C] glucose, as appropriate. (Each experiment represents or is an average of 2 or 3 similar experiments.) Herpes DNA was extracted by Marmur's method (3). Intact virus was treated with RNase just preceding the fourth chloroform:isoamyl alcohol (24:1) extraction. Viral DNA was hydrolyzed with DNase (0.5 mg DNase/ml sample, 0.1 ml 0.5 M $MgCl_2$, incubated 1 hr at 37° then 1 hr at 22°) followed by venom phosphodiesterase (Worthington Biochemical Corp.) hydrolysis. The reaction mixture contained 1 ml sample, 0.5 mg phosphodiesterase and 0.2 ml 0.2 M borate buffer (pH 8.5). Preparations were held 2 hr at 37° when additional enzyme (0.5 mg) was added to the above and samples were incubated another 2 hr at 37°. Acid hydrolysis involved refluxing samples 30 min in approximately 1 ml 0.1 M HCl/mg DNA of the original sample. Two dimensional paper chromatography using butanol-acetic acid-water (40:10:50) for the first direction and butanol-water (86:14) as the second solvent (4) was used to isolate deoxyribose.

Expt. No.	Labeled Compound	Viral Inoculum: Infective Centers	Total Cells Initially	μmoles Deoxyribose Isolated	Specific Activities counts/min/μmole Deoxyribose Isolated	Glucose in Media
1	[6-^{14}C] glucose	19	7.39×10^7	0.62	69,208	82,520
2	[1-^{14}C] glucose	9	4.54×10^8	1.05	5,311	100,000
3	[2-^{14}C] glucose	18	4.34×10^8	0.64	23,778	61,600

then washed twice in dilute saline-citrate-EDTA buffer in order to reduce contamination by host material and to chelate magnesium ions for DNase inhibition. Herpes DNA was extracted by the method of Marmur (3). Hydrolysis of viral DNA was achieved by enzymatic and chemical procedures as described in Table I. Deoxyribose was separated by the 2 dimensional paper chromatographic method of Partridge (4), while base ratios were determined by the procedure of Wyatt and Cohen (5) as employed by Astrachan and Volkin (6). Nucleic acid measurements based on extinction coefficients were calculated and deoxyribose was quantitated by the modified method of Burton (8).

RESULTS AND DISCUSSION

Data demonstrating isotope incorporation (Packard Tricarb liquid scintillation spectrometer, 85% efficiency, quenched samples) from [6-^{14}C] glucose and [2-^{14}C] glucose into deoxyribose of herpes DNA is shown in Table I; [1-^{14}C] glucose is used in the control system. By theoretical considerations one expects less isotope from glucose labeled in the 1 positions to be incorporated if pentose shunt activity is a principal energy yielding mechanism. Since these are 26 hr growth experiments it is reasonable to assume that CO_2 fixation and other mechanisms will allow some isotope incorporation from [1-^{14}C] glucose. Specific activities of deoxyribose isolated from viral infected systems where [6-^{14}C] glucose or [2-^{14}C] glucose serve as the chief energy source are much higher than pentose obtained from [1-^{14}C] glucose grown cultures. The fact that specific activities of isolated deoxyribose are lower than specific activities of the radioactive glucose added initially, suggests that glucose can be the source of viral DNA deoxyribose, with the possibility that some labeling may be found in purine and pyrimidine bases of viral DNA.

Table II records information regarding isotope incorporation into purine and pyrimidine bases of herpes DNA. Samples examined are aliquots of the same material as used for pentose isolations reported in the first table. Rf values are appropriate for the 4 bases as chromatographed in isopropanol-HCl-water solvent. Also mole % ratios which are high in guanine and cytosine and

low in adenine and thymine are typical for herpes DNA (9), while H. Ep. 2 cellular DNA (2) and mammalian DNA (10) in general are known to be high in adenine and thymine. Specific activities indicate that some isotope does enter the bases, but on a very small scale as compared with isotope content of the pentose moiety of viral DNA. We do not know if deoxyribose arises by the reductive pathway alone or if both oxidative and non-oxidative limbs of the pentose cycle are involved. The extent of randomization when pentose

TABLE II

ISOTOPE INCORPORATION FROM RADIOACTIVE GLUCOSE

INTO PURINE AND PYRIMIDINE BASES OF HERPES SIMPLEX DNA

Experiments, culture conditions, and extraction procedures were the same as those reported in Table I. Purine and pyrimidine bases of herpes DNA were characterized by descending paper chromatography in isopropanol:HCl:water (85:22:18) solvent following formic acid hydrolysis (5, 6).

Expt. No.	Labeled Compound	Viral Inoculum: Infective Centers	Base	Rf	µmoles Base	Mole Percent Ratio	Counts/ min	Specific Activities Counts/ min/µmole
1	[6-^{14}C] glucose	19	G*	0.18	2.38	33.4	1,222	513
			A*	0.33	1.11	15.6	547	493
			C*	0.47	2.53	35.5	2,725	1,077
			T*	0.72	1.10	15.6	2,315	2,104
2	[1-^{14}C] glucose	9	G	0.26	4.45	37.6	2,063	464
			A	0.35	1.93	16.3	759	393
			C	0.43	3.37	28.4	706	209
			T	0.65	2.10	17.7	632	301
3	[2-^{14}C] glucose	18	G	0.25	4.03	33.6	957	237
			A	0.34	1.78	14.8	435	244
			C	0.41	4.30	35.8	581	135
			T	0.62	1.90	15.7	435	229

*G = guanine; A = adenine; C = cytosine; T = thymine.

and glucose are subjected to prolonged equilibration, especially if pentose is not used as rapidly as it is formed, has not been ascertained. According to Sable (11), not only are nucleic acids important in regulatory functions of the cell, but they actually compose 20% of the total mass of some cells. Therefore, to study biosynthesis of pentose one learns about a large portion of cell synthetic activity. Data presented above demonstrate that deoxypentose of herpes DNA is derived from glucose, and that deoxypentose is the major labeled component when viral infected systems are grown 26 hr at 35° in the presence of [2-^{14}C] glucose or [6-^{14}C] glucose, but not in the presence of [1-^{14}C] glucose. Degradation of radioactive deoxyribose, in order to identify labeling patterns of the 5 carbons, will be of interest in predicting conversion pathways. When such pathways are learned with certainty the facts may be useful to those concerned with control of viral carcinogens.

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