INCREASE OF URIDINE KINASE ACTIVITY AFTER INFECTION OF CELLS WITH VACCINIA AND ROUS SARCOMA VIRUSES

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Using 4,5-C^{14}-6-azauridine /AzUR/ as substrate a marked enhancement of uridine kinase was found in many human tumours /Winkler et al., 1962; Kára et al., 1963/. However, the relationship supposed between the enhancement of this enzyme activity and the sensitivity of the tissue growth to the inhibitory effect of AzUR could not be confirmed. In the study of enzymatic activities after infection with poxviruses, an increase in the activities of several nucleoside phosphorylating enzymes was also described /Kit et al., 1962; McAuslan and Joklik, 1962/.

In present communication an enhancement of uridine kinase after infection of the cells with vaccinia and Rous sarcoma /RSV/ viruses is described. AzUR was shown to be a potent inhibitor of multiplication of these two viruses /Rada et al., 1960; Rada et al., in press/. Vaccinia virus derived from calf lymph preparation /Rada and Blaškovič, 1961/ and strain Prague of RSV were used. Experiments with vaccinia virus were carried out in chorioallantoic membrane cultures in vitro /Tamm et al.,

1953; Overman and Tamm, 1957/. After 18 hours' cultivation with continuous horizontal shaking, the cultures of chorioallantoic membranes /CAM/, noninfected as well as infected /multiplicity 5/, were washed and disrupted in a glass-homogenisator. The homogenate was centrifuged at 60,000 g for 30 minutes; the uridine kinase activity was determined in the supernatant. One experimental group contained parts of CAM with about 1.5×10^8 cells. Aliquot parts of CAM from one chick embryo were given in both groups - control as well as experimental.

In experiments with RSV the monolayers of chick embryo cells were cultivated in Petri dishes /Temin and Rubin, 1958/ and infected with RSV /multiplicity 0.01/. 7 days after infection the cells were scraped off, washed, homogenised and dealt with similarly as the cultures infected with vaccinia virus. In the other part of experiments with RSV, tumours from the breast muscle of chickens were used. Tumours as well as noninfected breast muscles /control/ were homogenised and dealt with further for estimation of the uridine kinase activity.

Uridine kinase activity was determined using 4,5-C^{14}-6-AzUR as substrate. Cell-free extracts were incubated with C^{14}-AzUR in the presence of Mg^{2+}-ions and ATP in a suitably buffered medium. After 25 minutes' incubation /at 37°C/ the mixture was deproteinized by heating to 100°C for 3 minutes and then centrifuged. The supernatant was separated by paper electrophoresis at 1000 V for 2 hours in 0.05 M ammonium formate buffer /pH 3.5/. The radioactivity of eluates was measured. The percent of AzUMP formed from the whole added amount of AzUR indicated the uridine kinase activity.

After infection with vaccinia virus a twofold increase of uridine kinase activity was found. In the case of infection with RSV zthe phosphorylating activity was higher. Cell-free extracts of both - infected tissue culture and the tumour formed in vivo - caused conversion of AzUR to AzUMP in 4 - 5%. Control materials were not shown to possess measurable conversion of AzUR to AzUMP /Table 1/.

Table 1

Activity of uridine kinase of normal cells and cells infected with vaccinia virus and RSV

Virus	Tissue	Incubation time	Percent of conversion of AzUR to AzUMP	
			Noninfected	Infected
Vaccinia	CAM	18 hours	2.8	5.6
RSV	Chick embryo cells	7 days	0	4.2
RSV	Muscle	11 days	0	5.2

Relatively low uridine kinase activity, in the case of vaccinia virus infection, might be connected with the system used. The activities also of other enzymes would in surviving CAM cultures presumably not be as high as in multiplying cells in monolayers. In exponentially growing HeLa cells, increases of up to 10 - 15-fold of enzymes phosphorylating dT and dU were found after infection with poxviruses /McAuslan and Joklik, 1962/. In the present experiments similarly as in another previous

communication, where thymidine phosphorylating activity was studied /Kit et al., 1962/, it was found that control noninfected tissue cultures lost the enzymatic activity if they were cultivated for a period of several days.

In most of the human tumour tissues tested uridine kinase activity was found to be higher than in the tissue from which the tumour was derived. However, only several of these tumours were shown to be susceptible to the inhibitory effect of AzUR. The supposition that enhancement of uridine kinase activity could be a general indicator of the susceptibility to the effect of AzUR was thereby not confirmed. On the other hand, in cells infected with vaccinia virus or RSV the increase of uridine kinase activity, due to virus infection, could be the cause of the increased formation of 6-azauridine-5'-phosphate. This anomalous nucleotide inhibiting the orotidylic acid decarboxylase, and thereby the biosynthesis of precursors necessary for virus synthesis, could be responsible for the selective inhibition of multiplication of these two viruses.

The question of the possible different character of uridine kinase in vaccinia virus or RSV infected cells from the enzyme performing the same function in noninfected cells will be the subject of further work. It has been shown previously that deoxyribonucleotide kinases, with properties quite distinct from those found in normal cells, are formed after infection of bacteria with T2 bacteriophage /Bello et al., 1961; Bessman and Bello, 1961/ or after infection of cells with pseudorabies virus /Nohara and Kaplan, 1963/.

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