HEXOKINASE ACTIVITY IN THE SOLUBLE NUCLEAR FRACTION AND CYTOPLASM OF CELL CULTURES INFECTED WITH HUMAN A6 AND A12 ADENOVIRUSES

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Hexokinase (EC 2.7.1.1) activity was studied in the soluble nuclear fraction and cytoplasm of rat embryonic fibroblasts (primary cultures) in the course of contact with infectious (A6) and oncogenic (A12) human adenoviruses. Higher activity of the enzyme was observed in the cytoplasmic fraction than in the nuclear in both normal and experimental samples. For the action of the type 6 infectious adenovirus this activity did not differ significantly from normal. Sharp changes in enzyme activity were observed on contact between the cells and the oncovirus. They occurred both in the nucleus and in the cytoplasm on the first days after infection with the virus, they gradually increased with an increase in the period of infection, and they reached their highest values on the 18th and 24th days. These disturbances were more marked in the cytoplasm than in the nucleus. It is postulated that these changes may be connected with uncontrollability of the process of cell proliferation.

KEY WORDS: rat embryonic fibroblasts; hexokinase; human adenoviruses.

A comparative study of interaction of infectious and oncogenic viruses with the cell is an important problem in modern oncovirology, for it is an approach that can shed some light on the possible specific character of differences in the expression of the cell genome.

Investigation of the changes in energy metabolism during transformation of cells is interesting since the reconstruction and redistribution of isoforms which participate in such transformation take place immediately after primary contact between virus and cell [1, 2].

The enzyme hexokinase (EC 2.7.1.1), which catalyzes the phosphorylation of glucose to glucose-6-phosphate and is one of the enzymes which determines the increased rate of glycolysis in tumors [7], has attracted particular attention.

The object of this investigation was to study the activity of nuclear and cytoplasmic hexokinase of cell cultures during primary contact with the cells of infectious and oncogenic viruses.

EXPERIMENTAL METHOD

Hexokinase activity was investigated in the soluble fraction of the nucleus and cytoplasm of primary trypsinized rat embryonic fibroblasts (REF). Tests were carried out under normal conditions and after infection with A6 adenovirus and A12 oncogenic virus. Trypsinized cultures were grown in 1.5-liter flasks in nutrient medium No. 199 with 10% bovine serum. On the third day after monolayer formation the culture was infected with the adenoviruses in titers of 4.5 log CPD_{56}/ml. The culture was tested on the first, third, fifth, eighth, 18th, and 24th days. An REF culture of the same age as the experimental samples served as the control.

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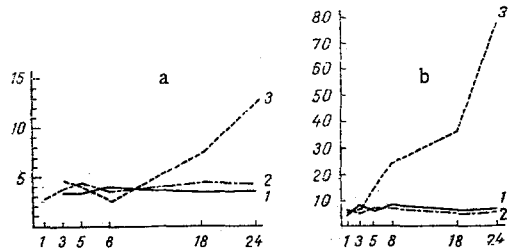

Fig. 1. Hexokinase activity in soluble fraction of nuclei and cytoplasm of cells of intact REF culture (1) and after infection with infectious A6 (2) and oncogenic A12 (3) human adenoviruses: a) nucleus; b) cytoplasm. Abscissa, time after infection with virus (in days); ordinate, enzyme activity (in μmoles glucose/g protein/min $\cdot 10^{-2}$).

The cell monolayer was removed with 0.02% versene solution, centrifuged at 110g for 15 min, and washed with physiological saline. Nuclei were isolated by the method of Chauveau et al., in the modification of Zbarskii and Georgiev [6]. The degree of purity was verified microscopically. The globulin fraction was extracted from the nuclei with 0.03 M Tris-glycine buffer in the cold [4, 6]. To obtain the cytoplasm the cells were homogenized in the cold in the same buffer (1 : 1) and allowed to stand for 15 h at 2°C, after which they were centrifuged at 750g for 30 min.

Total hexokinase activity in the resulting extracts was determined spectrophotometrically at 340 nm from the quantity of NADPH formed in medium containing 0.01 M potassium–phosphate buffer, pH 7.4, 5 mM NADP, 5 mM ATP, 5 mM $MgCl_2$, 0.4 IU glucose-6-phosphate dehydrogenase, and 5.5 mM glucose [6] and expressed in μmoles glucose/g protein/min.

EXPERIMENTAL RESULTS

Hexokinase activity in the soluble fraction of the cell nuclei of normal REF cultures was unchanged throughout the investigation, its mean value being $3.7 \cdot 10^{-2}$ μmole glucose/g protein/min. Total hexokinase activity in the cytoplasm of the normal culture was rather higher than in the soluble nuclear fraction. It remained virtually constant throughout the period of cultivation, with a mean value of $5.5 \cdot 10^{-2}$ μmole glucose/g protein/min.

In the nuclear fraction of REF from rats infected with infectious A6 adenovirus the hexokinase activity was almost indistinguishable from normal at all times of the investigation. The mean enzyme activity ($6.3 \cdot 10^{-2}$ μmoles glucose/g protein/min) in the cytoplasm likewise did not differ significantly from normal (Fig. 1) and hardly any change was observed in the course of time.

Increased hexokinase activity in the cytoplasm compared with the nuclear fraction in normal cultures and after infection with the A6 infectious adenovirus evidently reflects the higher level of glycolysis in the cytoplasm.

The study of the soluble fraction of the nucleus and cytoplasm of cells of an REF culture infected with oncogenic A12 adenovirus revealed substantial changes in enzyme activity. For instance, under the influence of the virus the nuclear hexokinase began to change on the third day of interaction between virus and cell. Activity of the enzyme was sharply increased in the soluble nuclear fraction on the 18th day, when loci of transformation (the beginning of morphological transformation) appeared in the cytoplasm (Fig. 1). Its activity was twice as high as the mean value both under normal conditions and in REF infected with A6 adenovirus. On the 24th day of interaction between virus and cell (when the culture was completely transformed) the total activity in the nucleus was 3.5 times greater than normal ($P < 0.001$) and 3.3 times greater ($P < 0.001$) than in REF infected with A6 adenovirus.

Total hexokinase activity in the cytoplasm of an REF culture infected with the oncogenic virus rose sharply on the fifth day of infection with the virus (by 2.6 times compared with normal and 2.2 times compared with REF infected with A6 adenovirus) (Fig. 1). A substantial increase of activity (by 6.6 times compared with normal and by 5.8 times compared with the cytoplasm of REF infected with A6 adenovirus) was discovered on the 18th day. On the 24th day of infection with oncogenic virus the cytoplasmic hexokinase activity was almost 14 times higher than normal and 12 times higher than in REF infected with A6 adenovirus. These findings

confirm the hypothesis of the increased potential capacity for glycolysis connected with malignant transformation of the cells [8].

Total hexokinase activity was much higher in the cytoplasm than in the nuclear fraction of the culture infected with oncogenic virus.

Hexokinase activity in cultures of normal REF cells in the nuclear fraction and cytoplasm thus did not change with time. The mean values of the enzyme activity in the cytoplasm were rather higher than in the nucleus. Under the influence of A6 infectious adenovirus the enzyme activity in the nucleus and cytoplasm showed no appreciable change. Sharp changes in hexokinase activity were observed during interaction between the oncogenic virus and the cell. They occurred both in the nucleus and in the cytoplasm after the first days of infection with the virus, they increased gradually with an increase in the period of infection, and reached their highest values on the 18th and 24th days.

The sharp changes in hexokinase activity in the nuclear fraction and cytoplasm of the REF culture transformed by oncogenic DNA-containing virus (compared with the infectious virus) in the course of time correlate with changes in the parameters of carbohydrate metabolism in the extracellular medium [5].

The writers [3] previously observed an increase in the activity of certain enzymes of glycolysis in the cytoplasm of REF cultures infected with an oncogenic variant of A12 adenovirus compared with cultures infected with variant A3, with weak oncogenic properties, and infectious A6 adenovirus.

The higher hexokinase activity in the cytoplasm than in the nuclear fraction after infection with infectious and, in particular, oncogenic viruses can probably be explained by the higher level of glycolysis stimulated in cells transformed by the oncogenic virus.

The results of these experiments thus established an increase in hexokinase activity in the soluble nuclear fraction and in the cytoplasm of REF cells in culture under the influence of DNA-containing oncovirus, indicative of the intensification of glycolytic processes characteristic of tissue undergoing malignant transformation. Consequently, the changes observed can be used as objective criteria of cell transformation.

The increase in hexokinase activity and the intensification of glycolysis in the early stages of transformation of REF cells under the influence of oncogenic virus can be presumed to be fundamental properties of malignant growth, possibly connected with the uncontrollability of the process of cell proliferation.

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