The authors are grateful to Professor V. Ya. Brodskii and to V. I. Fateev for valuable advice on measuring the thickness of cells.

LITERATURE CITED

1. A. F. Kuznetsova and V. Ya. Brodskii, Tsitologiya, 10, 392 (1968).
2. P. Bartels and G. Olson, in: Methods of Cell Separation, ed. N. Catsimpoolas, Vol. 3, New York (1980), pp. 1-100.
3. R. Baserga, Multiplication and Division of Mammalian Cells, Basel (1976).
4. R. Baserga and C. Nicolini, Biochim. Biophys. Acta, 458, 109 (1976).
5. P. Dean, Cell Tissue Kinet., 13, 299 (1980).
6. T. Kamata, S. Tanaka, and Y. Watanabe, Virology, 90, 197 (1978).
7. T. Kamata, S. Tanaka, and Y. Watanabe, Virology, 97, 224 (1979).
8. F. Kendall, F. Beltrame, and C. Nicolini, IEEE Trans. Biomed. Eng., 26, 172 (1979).
9. M. Musiani, F. Beltrame, M. Zerbini, et al., Microbiologica, 4, 101 (1981).
10. L. Trusal, A. Antony, and J. Docherty, J. Histochem. Cytochem., 23, 283 (1975).
11. W. Sawicki, in: Chromatin Structure and Function, C. Nicolini, ed., New York (1979), pp. 667-681.

EFFECT OF PREPARATIONS OF GLUTAMIN(ASPARAGIN)ASE FROM MICROORGANISMS ON DNA SYNTHESIS IN TUMOR CELLS

A. A. Pekhov, O. S. Zhukova,
T. P. Ivanova, V. A. Zanin,
T. T. Berezov, and Ya. V. Dobrynin

UDC 579.842.11:579.252.5]:579.257

KEY WORDS: glutamin(asparagin)ase; ovarian carcinoma; Fisher's lymphatic leukemia; DNA synthesis.

In cancer practice enzyme preparations possessing glutamin(asparagin)ase activity isolated from various microorganisms are used [1, 3-7]. However, the antiproliferative activity of preparations of this series depends on the sources from which they are obtained and the degree of purity. This paper gives comparative data on the effect of glutamin(asparagin)ase obtained from *Pseudomonas fluorescens* and *Pseudomonas boreopolis* 526 on DNA synthesis in cultures of HeLa-like human ovarian carcinoma cells (cells of line CaOv) and Fisher's lymphatic leukemia cells (line L-8).

EXPERIMENTAL METHOD

Two enzyme preparations of glutamin(asparagin)ase, isolated from different sources (the first was obtained from *Ps. fluorescens*, the second from *Ps. boreopolis* 526) and in their degree of purity were investigated. The enzyme obtained from *Ps. boreopolis* 526 was purified, as shown by disc electrophoresis, to a homogeneous state and had a specific activity of 89 I.U./mg protein as glutaminase and 71 I.U./mg protein as asparaginase. The enzyme obtained from *Ps. boreopolis* 526 was not homogeneous and had a specific activity of 40 I.U./mg protein as glutaminase and 29.5 I.U./mg protein as asparaginase. It follows from the values of specific activity given above that the enzymes differed somewhat in the ratio of their (glutaminase/asparaginase) activity: 1.25 for *Ps. fluorescens*, 1.46 for *Ps. boreopolis* 526.

The enzymes were purifed by traditional methods, which included salting out the protein with ammonium sulfate (45-90% saturation) from the protein extract, ion-exchange chromatography on DEAE-cellulose (grade DE-52), and gel-filtration. The CaOv cells were grown under standard conditions [2] in medium 199. The CaOv cells for the experiment were seeded in glass flasks (D = 2 cm) and grown for 24 h at 37°C. Each sample contained 200×10^3 to 300×10^3

Department of Biochemistry, P. Lumbumba Peoples' Friendship University, Moscow. All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 9, pp. 83-84, September, 1983. Original article submitted July 13, 1982.

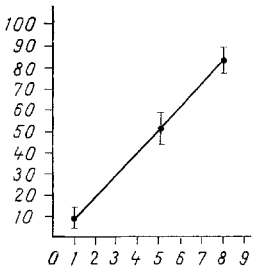

Fig. 1

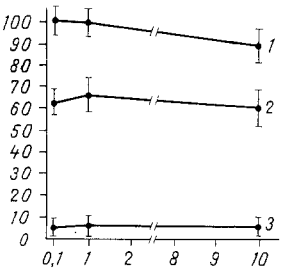

Fig. 2

Fig. 1. Effect of different concentrations of glutamin(asparagin)ase obtained from *Ps. fluorescens* on DNA synthesis in cells of line L-8. Abscissa, log (concentration, in I.U./ml); ordinate incorporation of [^{3}H]thymidine (in % of control).

Fig. 2. Effect of different concentrations of glutamin(asparagin)ase obtained from *Ps. fluorescens* and *Ps. boreopolis* 526 on incorporation of [^{3}H]thymidine in CaOv and L-8 cells. 1) Inhibition of DNA synthesis in CaOv cells in the presence of enzymes obtained from *Ps. boreopolis;* 2) the same for enzyme obtained from *Ps. fluorescens;* 3) inhibition of DNA synthesis in L-8 cells in the presence of enzyme obtained from *Ps. boreopolis* 526. Abscissa, concentration of preparations (in I.U./ml); ordinate, incorporation of [^{3}H]thymidine (in % of control).

cells in a volume of 2 ml. At the beginning of the experiments the enzyme preparation in a known concentration was added to the nutrient medium of the samples in minimal volume (20 µl) and incubated for 24 h at 37°C. The labeled precursor of DNA synthesis ([^{3}H]thymidine, specific activity 12 Ci/mmole) was added to the medium containing the samples 1 h before the end of the incubation time in a volume of 20 µl and in a final concentration of 1 µCi/ml. Nucleic acid synthesis was stopped by placing the samples in ice. The medium containing the samples was then poured off and the cells washed in Hanks' solution and with 2.5% $HClO_4$, and then hydrolyzed in 5% $HClO_4$ at 80°C for 20 min. The L-8 cells (a steady-state suspension culture of mouse leukemic cells was obtained from two DBA/2 males on the 12th day after intraperitoneal inoculation of strain I-5178Y by T. P. Ivanova et al.) were grown in medium RPMI-1640 containing 10% calf serum, 0.06% glutamine, and 0.008% gentamicin. Before the experiment the L-8 cells were seeded in glass flasks (D = 2 cm) at the rate of 200×10^3 to 300×10^3 cells in a volume of 2 ml. Preparations of the enzyme in minimal volume (10-20 µl) were added to the medium containing the samples, and incubated for 24 h at 37°C. The level of DNA synthesis was determined under the conditions indicated above. The samples were placed on ice 1 h after addition of [^{3}H]thymidine and centrifuged for 10 min (1200 rpm) and the cell residue was washed successively with Hanks' solution and 2.5% $HClO_4$ during centrifugation under the same conditions and hydrolyzed in 5% $HClO_4$ at 80°C for 20 min. Both types of digest were pooled, specimens of 0.1 ml were taken from each sample, added to ZhS-8 scintillation fluid, and counted on a Nuclear Chicago scintillation counter (USA). DNA synthesis in CaOv and L-8 cells was determined from the level of radioactivity in samples of hydrolysates and measured in cpm/sample. The experimental results are given as the arithmetic mean value calculated for 6-9 determinations ± the standard deviation. Differences between the values are significant at the $P \leqslant 0.05$ level.

EXPERIMENTAL RESULTS

As a result of exposure of the CaOv and L-8 cells with glutamin(asparagin)ase from *Ps. fluorescens* DNA synthesis was inhibited in both cases. Glutamin(asparagin)ase from *Ps. boreopolis* 526 in similar experiments inhibited DNA synthesis in cells of line L-8 but did not affect DNA synthesis in cells of line CaOv. Comparative data on the effect of both glutamin(asparagin)ases on incorporation of [^{3}H]thymidine into cells of line CaOv and L-8 are given in Figs. 1 and 2.

Incubation of the L-8 cells for 24 h with glutamin(asparagin)ase from *Ps. fluorescens* in a concentration of 1×10^{-1} I.U./ml led to complete suppression of DNA synthesis in the experimental samples compared with the control. Inhibition of [^{3}H]thymidine incorporation in the cells by the preparations in a concentration of 1×10^{-5} I.U./ml amounted to 50.2%. When the concentration of the preparation was low (1×10^{-8} I.U./ml), the degree of inhibition was 17.4% (Fig. 1).

Inhibition of DNA synthesis in CaOv cells in the presence of enzymes from *Ps. fluorescens* was independent of concentration (10, 1, and 0.1 I.U./ml) and was 36.8%-38.3% (Fig. 2, 2).

The study of the effect of glutamin(asparagin)ase from *Ps. boreopolis* 526 on incorporation of [^{3}H]thymidine in CaOv and L-8 cells showed that exposure of the cells with the preparation in concentrations of 10, 1, and 0.1 I.U./ml led to complete suppression of DNA synthesis in the second case but did not affect incorporation of [^{3}H]thymidine into the CaOv cells (Fig. 2, 3).

LITERATURE CITED

1. T. T. Berezov, Vestn. Akad. Med. Nauk SSSR, No. 11, 35 (1971).
2. Ya. V. Dobrynin, T. I. Monatova, and N. A. Kondrat'eva, Lab. Delo, No. 3, 143 (1974).
3. S. V. Manoilov, E. I. Orlova, and R. G. Polosova, Vopr. Onkol., No. 1, 64 (1966).
4. A. Ya. Nikolaev, Usp. Khim., 32, 1087 (1969).
5. B. Clarkson and G. Krakkoff, Cancer (Philadelphia), 25, 279 (1970).
6. J. Roberts, J. S. Holcenberg, and Y. Dolowy, Nature, 227, 1136 (1970).
7. J. Roberts and J. S. Holcenberg, J. Biol. Chem., 247, 84 (1972).

WEAKENING OF DNA—PROTEIN INTERACTIONS IN MOUSE LEUKEMIC LYMPHOBLASTS UNDER THE INFLUENCE OF SOME ANTITUMOR AGENTS

A. M. Serebryanyi, N. I. Sjakste, A. I. Gorin, P. I. Tseitlin, N. S. Bogomolova, V. A. Chernov, V. S. Shapot*, and A. V. Likhtenshtein

UDC 616.155.392-07:616.32-008.939.6

KEY WORDS: antitumor agents; leukemic lymphoblasts; DNA—protein interactions.

A method of testing DNA—protein interactions in eukaryote cells with the aid of nucleoprotein-celite chromatography (NPCC) was suggested previously [6, 7]. Sharp differences in the strength of DNA—protein interaction in resting and proliferating cells have been detected by the NPCC method: In the first case the cell DNA is eluted from the column under relatively mild conditions (1.5 M LiCl, 8 M urea, 4°C), whereas in the second case much more rigorous conditions are required (4 M LiCl, 8 M urea, 95°C). Actions leading to withdrawal of the cells from the division cycle cause transition of DNA from the form firmly bound with protein (form II) into a form relatively weakly bound with protein (form I). Exciting cells to divide causes the opposite transition. The use of the NPCC method to study changes in DNA—protein interactions which may arise in the deoxyribonucleoprotein complex (DNA complex) of eukaryotes after exposure of the cell to biologically active substances and, in particular, after the action of the well-known mutagen, carcinogen, and carcinolytic agent 1-nitroso-1-methylurea

*Corresponding Member, Academy of Medical Sciences of the USSR.

Sector of Kinetics of Chemical and Biological Processes, Institute of Chemical Physics, Academy of Sciences of the USSR. Laboratory of Biochemistry, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Laboratory of Molecular Biology, Institute of Medical Genetics, Academy of Medical Sciences of the USSR. Laboratory of Experimental Chemotherapy of Tumors, S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 9, pp. 84-86, September, 1983. Original article submitted November 19, 1982.