EFFECT OF THE DEGREE OF POLYMERIZATION OF EXOGENOUS DNA ON ITS INCORPORATION INTO RAT THYMOCYTES IN VITRO

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The kinetics of incorporation of exogenous homologous DNA into rat thymocytes in vitro were studied to examine the effect of its degree of polymerization on this process. Depending on the degree of depolymerization of the original preparation the pattern of incorporation kinetics can vary from steady to periodic. The rhythm of incorporation is accompanied by cyclic changes in the strength of the bond joining the exogenous material to the nuclei.

Administration of exogenous DNA leads to various biological and biochemical effects in animals [3]. Most attention has been paid to the radioprotective properties of DNA [7, 11]. A few studies of the kinetics of penetration of exogenous material have been published, but they were carried out only with high-polymer DNA [8]. However, there is reason to question the view that some effects are induced only by high-polymer preparations [11].

The object of this investigation was to study the effect of the degree of polymerization on the quantitative and qualitative characteristics of the kinetics of DNA incorporation into rat thymocytes.

EXPERIMENTAL METHOD

Thymocytes were obtained from the thymus glands of noninbred male albino rats weighing 100-120 g and suspended in Hanks's medium. The cell suspension in a concentration of 2.6×10^7 cells/ml was incubated for 120 min at 37°C with constant mixing. The state of the cells was monitored by eosin tests. Rat thymus DNA was obtained by the Kirby–Georgiev method [1]. The hyperchromic effect was 39-40%, contamination with RNA (orcine test) did not exceed 1%, and contamination with protein (Lowry's method) did not exceed 0.05%. The weighted-mean molecular weight (M), calculated from the characteristic viscosity, was 1×10^7 daltons. DNA was labeled in vivo [2] or in vitro [5]. The specific activity of the preparations was 4.0×10^2 pulses/min·μg. The labeled preparations were degraded by prolonged keeping at 4°C. A rough estimate of the limits of molecular weight of the fragments was obtained from the characteristic viscosity, using the two limiting exponents corresponding to M = 1.12 (a rigid coil) and M = 1.8 (a rigid rod). During incubation of the cells with DNA-H^3 (2.5 μg/ml) or thymidine (4.3×10^{-2} μg/ml) aliquot samples were taken from the mixture and treated in various ways [9] depending on the purpose of the individual experiments. A solution of DNA isolated from cells [13] or the nuclear fraction [4] was sealed in ampules and incubated at 100°C for 3 h to obtain total exchange of the tritium by hydrogen. The liquid from the ampules was treated by vacuum distillation and transferred quantitatively to a liquid scintillator (1 ml to 10 ml of a solution of 3 PPO, 0.3 POPOP, and 100 g naphthalene in 1 liter dioxan), after which the radioactivity was measured with a "Picker" scintillation counter. The DNA content in each sample was measured (from the spectophotometric data) and the final result expressed in pulses/min/mg.

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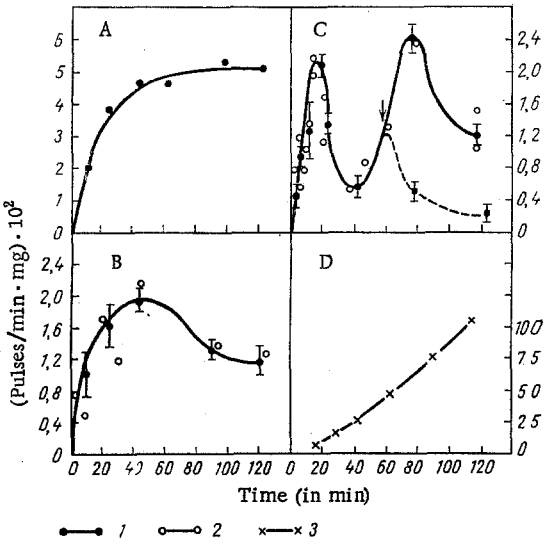

Fig. 1. Incorporation of DNA and its degradation products into cells (1) and nuclei (2). A) 10^7 daltons, B) 10^6 daltons, C) 10^5 daltons, D) thymidine (3). Arrow indicates time of addition of acid to incubation medium; in this case broken line shows direction of incorporation. Abscissa, incubation time (min); ordinate, quantity of label incorporated into cell DNA (pulses/min) × 10^2.

EXPERIMENTAL RESULTS

The kinetics of appearance of exogenous DNA in the composition of the DNA isolated from the recipient cells or nuclei is shown in Fig. 1. Clearly the type of kinetics of incorporation depended on the degree of polymerization of the original preparation and changed gradually from monotonous to extremal and periodic with an increase in the degree of fragmentation of the donor molecules (Fig. 1A, B, C). This rule did not extend to incorporation of precursors of DNA synthesis (Fig. 1D). The periodicity of incorporation (Fig. 1B, C) can arise only if the cells behave synchronously relative to the substrate. On the basis of the established dependence of the process on pH [9], the possibility of synchronization was demonstrated in principle by rapid addition of acid to the incubation medium (Fig. 1C). In that case the viability of the cells, determined by means of the eosin test, remained unchanged, indicating the reversibility of the process.

Agreement between the results of determination of labeled DNA in the composition of the cellular and nuclear DNA is in accordance with data in the literature [9] and shows that most of it is bound with the material of the nucleus and that the process is probably accompanied (Fig. 1B, C) by a change in the stability of the resulting complex [12]. Experiments accordingly were carried out to study incorporation of DNA into the cells followed by treatment of their nuclei with deoxyribonuclease (DNase). The results showed that the strength of the bond between substrate and nucleus varies at different stages of the process. For instance, if DNA preparations of different degrees of polymerization were used, DNase treatment was less effective at the time of the maximum of incorporation than the minimum. The periodicity of incorporation is thus accompanied by periodic changes in the strength of binding. The discovery of this relationship of extremal type between kinetics and degree of polymerization indicates that the character of the process is unconnected with the nature of the DNA. This hypothesis agrees with the observations of Schimizu et al. [14], who found an extremum for the incorporation of heterologous DNA although, unfortunately, its molecular weight was not established.

Considering the evidence for the formation of a complex of protein with exogenous DNA [6] and of its fragmentation in the recipient cells [10], on the appearance of acid-soluble products of exogenous DNA in the process of its incorporation, and also the results of the present experiments, it is postulated that interaction between the cell and exogenous DNA takes place in cycles and that each cycle consists of several stages: 1) incorporation into the nucleus with the formation of a reversible DNP complex; 2) depolymerization, accompanied by weakening of the bond fixing the exogenous material in the complex; 3) liberation of depolymerization products into the cytoplasm and medium. The duration of the cycle depends on the degree of polymerization of the original preparation, and it is the chief factor to be considered when the duration of a kinetic experiment is chosen.

LITERATURE CITED

1. I. B. Zbarskii et al., Chemistry and Biochemistry of Nuclei Acids [in Russian], Leningrad (1968).
2. K. N. Muksinova, R. E. Rusinova, and R. E. Libinzon, Radiobiologiya, No. 1, 69 (1971).
3. P. M. Bhargava and G. Shanmugam, Progr. Nucl. Acid Res., 11, 103 (1971).
4. J. Chauveau, J. Moule, and C. Roniller, Exp. Cell Res., 11, 317 (1956).
5. F. Dopper-Bernardi and G. Felsenfeld, Biopolymers, 8, 733 (1969).
6. W. J. Harris and G. C. Barr, Molec. Gen. Genet., 113, 331 (1971).
7. D. T. Kanazir, Progr. Nucl. Acid Res., 9, 117 (1969).
8. F. Laval and M. E. Malaise, C. R. Acad. Sci. (Paris), 268, 3119 (1969).
9. F. Laval, M. E. Malaise, and J. Laval, Exp. Cell Res., 63, 69 (1970).

10. S. Meizel and E. R. M. Kay, Biochim. Biophys. Acta, 103, 403 (1965).
11. D. Perrovic, A. Ferle-Vidovic, V. Habazin, et al., Int. J. Radiat. Biol., 18, 243 (1970).
12. P. L. Schnell, Biochim. Biophys. Acta, 166, 156 (1968).
13. G. Schmidt and S. J. Thannhauser, J. Biol. Chem., 161, 83 (1945).
14. T. Schimizu, S. Koyama, and M. Iwafuchi, Biochim. Biophys. Acta, 55, 795 (1962).