INCORPORATION OF DEOXYRIBONUCLEOTIDES INTO TERMINAL POSITIONS OF DNA

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Received August 4, 1961

An enzyme fraction from calf thymus nuclei which incorporates single ribonucleotides into terminal positions of DNA has been described (1, 2, 3). It has been found that this same enzyme fraction (referred to in this communication as 'nuclear enzyme') catalyses the incorporation of deoxyribonucleotides into terminal positions of DNA and that this activity appears to be distinct from that of DNA polymerase.

RESULTS

The 'nuclear enzyme' which incorporates deoxyribonucleotides into terminal positions of DNA can be distinguished from DNA polymerase by the fact that the incorporation of one deoxyribonucleotide into DNA is not enhanced by the presence of the complementary deoxyribonucleoside triphosphates in the incubation mixture. This characteristic has been used as a means of assay of the 'nuclear enzyme'.

The results in Table I show that incubation of the 'nuclear enzyme' with DNA and P^{32}-dCTP results in the incorporation of dCMP into DNA; when the dATP, dGTP and TTP are also present in the incubation mixture no enhancement of incorporation of P^{32}-dCMP into DNA is noted, but rather an inhibition of incorporation. This contrasts with the behavior observed with DNA polymerase (4, 5) and is also presented in this Table for purposes of comparison. When the 'nuclear enzyme' and calf thymus polymerase (5) are incubated together, the stimulation characteristic of DNA polymerase is observed, indicating that the enzymatic activity of the 'nuclear enzyme' is not an artifact imposed upon a DNA polymerase present in this fraction by contaminating enzymes.

TABLE I

Effect of the 'Nuclear Enzyme' on the Activity of DNA Polymerase

Enzyme added	Precursor A: P^{32}-dCTP incorporation cpm	Precursor B: P^{32}-dCTP plus dATP, dGTP and TTP incorporation cpm	Ratio A/B
Nuclear enzyme	236	111	2.1:1
DNA polymerase	247	3200	1:13
Nuclear enzyme plus DNA polymerase	406	4060	1:10

The complete system contained (in 1.0 ml): 10 µmoles of phosphate (K^+, pH 7.2), 10 µmoles of $Mg^{++}(Cl^-)$, 10 µmoles of cysteine, 200 µg. of heated calf thymus DNA, 60 mµmoles of P^{32}-dCTP(P^{32}-P-P) and an equivalent amount of each the non-radioactive deoxyribonucleoside triphosphates where indicated; 'Nuclear enzyme' and DNA polymerase (prepared by the method of Bollum from calf thymus) (5) were added to the appropriate tubes. Incubation time: 60 minutes. Determination of incorporation into DNA was performed by published methods. (6)

During the course of purification of this enzyme fraction from calf thymus nuclei, the original preparations contain DNA polymerase as indicated in Table II (Fraction A and Fraction B) by the stimulation of incorporation of P^{32}-dCMP by the presence of the complementary deoxyribonucleoside triphosphates. When Fraction B is adsorbed onto a DEAE-cellulose column, elution of the column with 0.02 M potassium phosphate, pH 7.2 removes the 'nuclear enzyme', no enzymatic activity can be recovered by elution of the column with 0.06 M potassium phosphate, pH 7.2 whereas subsequent elution with 0.06 M potassium phosphate, pH 7.2 plus 0.1 M $(NH_4)_2SO_4$ results in the removal of the DNA polymerase from the column, as evidenced by the stimulation of P^{32}-dCMP incorporation into DNA by the complementary deoxynucleoside triphosphates. These results indicate that the two enzymatic activities are due to distinct entities.

It has also been found that the type of incorporation observed in the presence of the 'nuclear enzyme' appears to be limited to the incorporation of a few deoxyribonucleotides to the ends of DNA chains and not to an extensive polymerization of DNA. This has been established by preparing dCMP^{32}-DNA and

TABLE II

Separation of 'Nuclear Enzyme' from DNA Polymerase

Fraction added	Incorporation in presence of P^{32}-dCTP(P^{32}-P-P) mumoles	Incorporation in presence of P^{32}-dCTP, dATP dGTP, TTP mumoles
Nuclear pellet (Fraction A)	0.26	0.64
Extract of Fraction A (Fraction B)	0.46	0.89
0.02 M K Phosphate	0.38	0.24
0.06 M K Phosphate	0.01	0.01
0.06 M K Phosphate plus 0.10 M Ammon. Sulfate	0.12	0.39

The incubation conditions were as described in the legend to Table I.

following the release of radioactivity and total nucleotides from the product DNA into acid soluble form on hydrolysis with snake venom phosphodiesterase (7). The results presented in Table III indicate that when P^{32}-dCTP was the sole precursor present during the incorporation reaction, almost 90% of the incorporated radioactivity was released within the time required to render acid soluble 5% of the DNA. When a full complement of deoxynucleoside triphosphates (P^{32}-dCTP, dATP, dGTP, and TTP) were present during the incorporation reaction, more then 50% of the incorporated radioactivity was released from the product DNA within the time required to render acid soluble 5% of the DNA. Similar data have also been obtained with TMP^{32}-DNA (and C^{14}-GMP-DNA) and also indicate that the 'nuclear enzyme' catalyzes a limited addition of deoxynucleotides (or ribonucleotides) to the ends of preexisting DNA chains.

References

1. Krakow, J. S., and Kammen, H. O., Federation Proc. 19, 307 (1960).

2. Krakow, J. S., and Canellakis, E. S., Federation Proc. 20, 361 (1961).

TABLE III

Action of Venom Phosphodiesterase on Product DNA

Time minutes	Radioactivity % released: Product prepared with: $dCTP^{32}$ alone	$dCTP^{32}$ plus dATP, dGTP, TTP	Optical Density % released
10	18	6	<1
20	33	17	1
40	50	26	2
90	74	41	3
180	89	54	5

Labeled product-DNA was prepared as indicated in Table I for 'nuclear enzyme' and the labeled DNA (1 mg) isolated. The product was treated with snake venom phosphodiesterase (7) and the radioactivity and optical density at 260 mμ rendered acid soluble were determined.

3. Krakow, J. S., Kammen, H. O., and Canellakis, E. S., Biochim. Biophys. Acta, in press.

4. Bessman, M. J., Lehman, I. R., Simms, E. S., and Kornberg, A., J. Biol. Chem., 233, 171 (1958).

5. Bollum, F. J., J. Biol. Chem. 235, 2399 (1960).

6. Mantsavinos, R., and Canellakis, E. S., Cancer Res. 19, 1239 (1959).

7. Adler, J., Lehman, I. R., Bessman, M. S., Simms, E. S., and Kornberg, A., Proc. Natl. Acad. Sci., U. S., 44, 641 (1959).