SEPARATION OF THE ENZYME CATALYZING POLYMERIZATION OF DEOXYRIBONUCLEOSIDE DIPHOSPHATES FROM THE PREPARATIONS OF *ESCHERICHIA COLI* DNA POLYMERASE I

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1. Introduction

DNA repair is considered to be the main biological function of *Escherichia coli* DNA polymerase I [1]. Besides some other catalytic properties of the enzyme have been found. Highly purified preparations of the enzyme catalyze phosphorylation of deoxyribonucleoside diphosphates [2]. *Escherichia coli* DNA polymerase I is able to carry out RNA-dependent synthesis of DNA [3–5]. The enzyme catalyzes de novo synthesis of poly(dA-dT):poly(dA-dT) [6] and poly(dG):poly(dC) [7].

We have found that preparations of *E. coli* DNA polymerase I are able to catalyze template- and primer-independent synthesis of polydeoxynucleotides from deoxyribonucleoside diphosphates. The data presented in this paper demonstrate that this property does not belong to *E. coli* DNA polymerase I itself but is an inherent characteristic of an independent enzyme. The presence of the enzyme in the preparations of *E. coli* DNA polymerase I can explain the ability of the latter to catalyze de novo synthesis of poly(dG):poly(dC).

2. Materials and methods

DNA polymerase I was isolated from *E. coli* MRE 600 as in [8] with the exception that DEAE cellulose chromatography was performed as in [9]. DNA polymerase activity was assayed as in [9].

d[^{3}H]NDP and d[^{3}H]NTP were prepared from commercial preparations of deoxy[^{3}H]nucleosides using a nonspecific carrot phosphotransferase [10] and *E. coli* nucleotidyl kinase [11]. The specific activity of the preparations of d[^{3}H]NDP and d[^{3}H]NTP was 10–20 Ci/mmol.

Unlabelled deoxyribonucleoside triphosphates and diphosphates were purchased from Special Bureau of Design and Technology of Biologically Active Substances (Novosibirsk, USSR); ATP from Reanal (Hungary); labelled deoxyribonucleosides (10–20 Ci/mmol) from Isotop (USSR). The preparation of deoxynucleotidyl kinase was a gift of Dr E. A. Vasyunina.

Products of dNDP polymerization were studied by DEAE-cellulose microcolumn chromatography [12].

The activity of dNDP-polymerizing enzyme was estimated by the conversion of d[^{3}H]NDP into an acid-insoluble product and by using DEAE-cellulose microcolumn chromatography. The incubation mixture is described in the legend to fig.1.

3. Results

3.1. *Separation of the enzyme*

We have established that fractions V and VII of *E. coli* DNA polymerase I obtained as in [8] are able to catalyze the unprimed synthesis of polydeoxynucleotides from dNDP. Using DEAE-cellulose chromatography we have succeeded in separation of the protein possessing this activity from DNA polymerase I. The activity was eluted between 0.14 M and 0.2 M potassium phosphate while DNA poly-

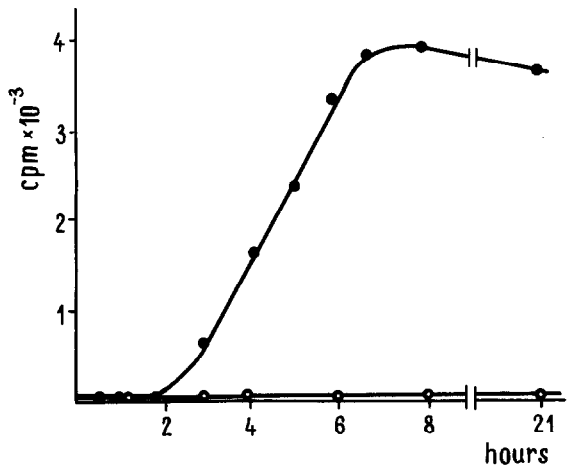

Fig.1. The kinetics of the polymerization of dADP. The incubation mixture (0.1 ml) contained 0.05 μmol dADP and d[^{3}H]ADP (10 000 cpm); 0.1 μmol $MgCl_2$; 10 μmol potassium phosphate (pH 8.5) and 1–2 μg enzyme preparation. After incubation at 37°C the reaction was stopped by cooling and by addition of 0.1 ml bovine serum albumin solution (4 mg/ml) and 0.2 ml 1 M perchloric acid at 0°C. The precipitate was collected by filtration through nitrocellulose filters (Sinpor, Czechoslovakia); radioactivity was counted in a liquid scintillation spectrometer (Mark-II, Nuclear Chicago, USA). The incubation conditions in [14] were used for studying the reaction during the lag period. When ADP was tested as substrate the reaction mixture contained 0.1 μmol [^{3}H]ADP (10 000 cpm) and 0.5 μmol $Mg(CH_3COO)_2$ in 0.1 ml 0.1 M Tris–HCl (pH 8.1). (–•–) d[^{3}H]ADP; (–○–) [^{3}H]ADP.

merase was eluted between 0.14 M and 0.17 M potassium phosphate. The fractions eluted between 0.17 M and 0.2 M potassium phosphate were pooled and filtered through an XM-50 Amicon ultrafilter. The filtrate was free of DNA polymerase I activity but retained the ability of de novo synthesis of polydeoxynucleotides from dNDP.

By using the modified procedure for the isolation of DNA polymerase I [13] it was possible to obtain the DNA polymerase I unable to provide polynucleotide synthesis de novo.

3.2. *Properties of the enzyme*

It was shown that the enzyme was able to polymerize dADP, dGDP, TDP (dCDP was not examined). The main properties of the enzyme were studied using dADP as a substrate.

The reaction of dNDP polymerization starts after a lag period which varies from 2–20 h for different preparations of the enzyme (fig.1). When dADP was replaced by ADP no formation of acid insoluble product was observed (fig.1, table 1). The data in [2] suggest that dNDP can be phosphorylated to dNTP and the latter can serve as a real substrate for polymerization. However when phosphorylation of d[^{3}H]NDP to d[^{3}H]NTP was stimulated by the addition of ATP to the reaction mixture, the polymerization was even inhibited (table 1). Finally when d[^{3}H]ADP was replaced by d[^{3}H]ATP, the reaction

Table 1
Substrate specificity of the enzyme

Exp. no.	Experimental conditions	Labelled nucleotide incorporation into acid-insoluble product (cpm)
1	d[^{3}H]ADP	6000
2	[^{3}H]ADP	170
3	d[^{3}H]ADP+ATP	190
4	d[^{3}H]ATP	300

In exp. 1, the reaction mixture contained 0.1 μmol dADP and d[^{3}H]ADP (14 000 cpm); in exp. 2, 0.1 μmol [^{3}H]ADP (10 000 cpm); in exp. 3, 0.1 μmol d[^{3}H]ADP and 10 μmol ATP; in exp. 4, 0.1 μmol of dADP and d[^{3}H]ATP (200 000 cpm). Otherwise the experimental conditions are identical to those described in the legend to fig.1

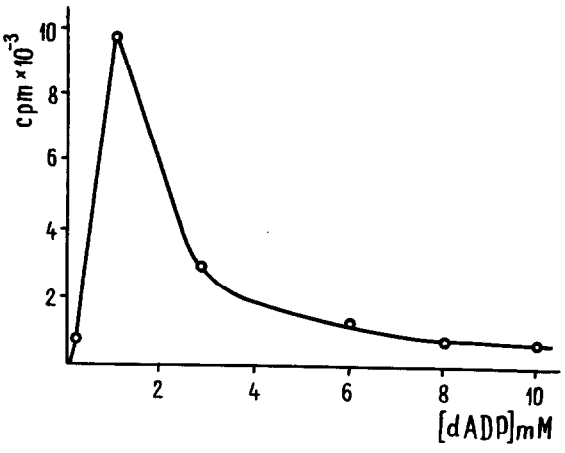

Fig.2. The dependence of the polymerization extent on the dADP concentration. Experimental conditions as in fig.1 legend.

Table 2
Investigation of products of the enzymatic polymerization of dADP and dGDP

NaCl (M)	Eluted oligonucleotide length	% oligonucleotides of different length	
		poly(dA)	poly(dG)
0.15–0.27	5–10	31	6
0.27–0.4	10–50	56	50
1.0	> 100	13	44

The reaction mixture contained either d[^{3}H]ADP (14 000 cpm) or d[^{3}H]GDP (14 000 cpm). Otherwise the composition of the reaction mixture was the same as described in the legend to fig.1. Incubation time was 12 h at 37°C; 10 μl aliquots were applied to DEAE-cellulose column (25 μl). Elution was carried out with 600 μl linear gradient of NaCl from 0–0.4 M in 7 M urea (flowrate 0.6 ml/h). Absorption was measured at 260 nm

of polymerization did not take place. The data suggest that deoxyribonucleoside diphosphates are the only substrates used by the enzyme, and they can not be replaced by NDP or dNTP.

The reaction was activated by Mg^{2+}, the optimal concentration of which was 1 mM but reaction could proceed without any divalent cations.

As it is shown in fig.2, when the concentration of substrate exceeded 10^{-3} M the inhibition of the polymerization occurred. It was shown that the inhibition was not due to the lack of Mg^{2+} cations.

The pH optimum of the reaction was at 8.5.

The estimation of the polymerization products length showed that the amount of short oligonucleotides varied when different dNDP were used as substrates (table 2).

3.3. *Probable role of the enzyme in poly(dG):poly(dC) synthesis*

The ability of the enzyme preparations of *E. coli* DNA polymerase I to carry out de novo synthesis of poly(dG):poly(dC) can be explained by the presence of the enzyme which catalyzes dNDP polymerization. Poly(dG):poly(dC) synthesis provided by the DNA polymerase I proceeds after a lag period comparable to that observed for the reaction of dNDP polymerization.

When the DNA polymerase I preparation was incubated for 20 h with dGTP which contained 1–5% dGDP, the formation of oligonucleotides was observed. Perhaps these small admixtures of dGDP and dCDP are sufficient for oligo(dG) and oligo(dC) synthesis.

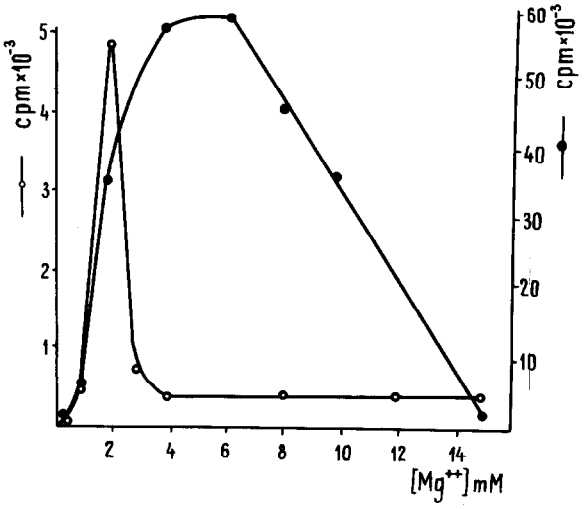

Fig.3. The dependence of the de novo synthesis of poly(dG):poly(dC) synthesis on Mg^{2+} concentration. The reaction mixture contained in 0.1 ml 0.025 μmol dGTP; 0.025 μmol dCTP; d[^{3}H]GTP (80 000 cpm); 6 μmol potassium phosphate (pH 6.5) and 2 units DNA polymerase I (DEAE cellulose fraction with spec. act. 300 units/mg). Incubation was performed at 37°C. Incorporation of d[^{3}H]GMP was measured after 6 h (–o–) and 20 h (–•–).

These oligonucleotides can serve as efficient templates and primers for the DNA polymerase I. This explanation is consistent with the observed dependence of the rate of poly(dG):poly(dC) synthesis on Mg^{2+} concentration (fig.3). It appeared that at the end of the lag period $1-2 \times 10^{-3}$ M Mg^{2+} concentration was optimal, just as for the enzymatic dNDP polymerization. Twenty hours after the beginning of the incubation the optimum of the Mg^{2+} concentration rises to $6-7 \times 10^{-3}$ M, which is characteristic for *E. coli* DNA polymerase I.

4. Discussion

The data presented demonstrate that the enzyme catalyzing de novo synthesis of polydeoxynucleotides from dNDP can be separated from *E. coli* DNA polymerase I. We suggest to name it deoxynucleoside diphosphate:oligonucleotide deoxynucleotidyl transferase (catalyzing de novo polymerization), or in abbreviated form, dNDP-deoxynucleotidyl transferase.

An enzyme from *E. coli* which also catalyzes dNDP polymerization was isolated [14]. However this enzyme polymerized NDP more efficiently than dNDP [15,16]; that was the reason to identify the enzyme as polyribonucleotide phosphorylase. In contrast to the enzyme isolated [14], dNDP-deoxynucleotidyl transferase is not able to polymerize ribonucleoside diphosphates under conditions optimal for polynucleotide phosphorylase. The extent of dNDP polymerization by dNDP-deoxynucleotidyl transferase reaches 40–60%, while the extent of the reaction catalyzed by the enzyme [14] does not exceed 1–5% [14–16]. For the reaction catalyzed by the enzyme [14] Mn^{2+} is essential and it can not be replaced by Mg^{2+} [14], while dNDP-deoxynucleotidyl transferase is activated by Mg^{2+}.

Data suggest that dNDP-deoxynucleotidyl transferase is a new enzyme distinct of the enzyme isolated from *E. coli* [19] and of *E. coli* DNA polymerase I. The biological functions of the enzyme remain obscure. It seems probable that dNDP-deoxynucleotidyl transferase can act as terminal deoxynucleotidyl transferase [17]. It is tempting to speculate that the enzymes with such properties can participate in adding 'sticky' ends to DNA fragments. In this way they can be used for natural 'gene engineering' for the integration of genetic material in bacteria or animal genomes.

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