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ACTIVATION OF MAMMALIAN DNA LIGASE BY POLYAMINES

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SUMMARY: The activity of calf thymus DNA ligase was stimulated 2- to 3-fold by the addition of putrescine, spermidine and spermine. The optimal concentrations were 5 mM for putrescine, 0.5 mM for spermidine and 0.05-0.1 mM for spermine. Spermidine not only lowered the apparent Km value for Mg^{2+} but also elevated the V value, whereas the apparent Km value for ATP was not changed. In the presence of Mn^{2+} as a divalent cation, the V value was stimulated 3-fold by 0.5 mM spermidine. Monovalent cations, such as KCl and NaCl could replace the polyamines at the optimal concentrations of 50 mM and diminish the stimulation by the polyamines.

INTRODUCTION

Accumulating lines of evidence indicate that polyamines, whose concentrations are high in actively proliferating animal tissues, may play an important role in the syntheses of macromolecules including DNA [1,2]. DNA ligases which covalently join single-stranded breaks in duplex DNA have been partially purified from mammalian cells and tissues [3-12]. In the course of our studies on the function and regulation of mammalian DNA ligase, the level of the enzyme activity has been shown to increase in regenerating rat liver, rat hepatoma, rat kidney tumor, human hepatoma and developing rat brain with increase in DNA synthesis [13-18]. In this communication we describe the effect of polyamines and salts on the activity of DNA ligase highly purified from calf thymus.

MATERIALS AND METHODS

Reagents and Preparations. [γ-^{32}P]ATP was obtained from the Radiochemical Centre (Amersham, England) or prepared by the method of Walseth and Johnson [19]. Calf intestine alkaline phosphatase (grade I) was purchased from Boehringer (Mannheim, F.R.G.). Spermidine·3HCl and spermine·4HCl were obtained from Sigma (St. Louis, U.S.A.) and putrescine·2HCl from Nakarai Chemicals

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(Kyoto, Japan). Blue-Sepharose CL-6B was purchased from Pharmacia (Uppsala, Sweden). GF/A filter paper was obtained from Whatman (Kent, England). Other reagents and preparations were described previously [12,20].

Assay of DNA Ligase. The activity of DNA ligase was assayed by the method described previously [12] with significant modifications. The standard reaction mixture (0.2 ml) contained 5-15 μg [5'-^{32}P]nicked DNA (1-2 x 10^4 cpm/5-20 pmol)/15 mM $MgCl_2$/0.2 mM ATP/2 mM dithiothreitol/20 μg bovine serum albumin/75 mM Tris-HCl pH 7.5/enzyme (0.04-0.1 μg protein). The mixture was incubated at 37°C for 10 min and then 0.2 ml of carrier DNA solution (0.25 mg/ml) and 0.4 ml of chilled 10% trichloroacetic acid were added in an ice-water bath. After 5 min, the precipitate was collected by a 10-min centrifugation at 2000 x g, washed once with 1.5 ml of 10 mM HCl and completely dissolved in 0.2 ml of 0.1 M NaOH followed by the addition of 0.1 ml of 0.3 M Tris-HCl buffer pH 7.0. The solution was incubated at 37°C for 30 min in the presence of 0.5 unit of alkaline phosphatase. To the solution 0.1 ml of 0.1 M sodium pyrophosphate and 3 ml of chilled 5% trichloroacetic acid were added. After 10 min at 0°C, the resulting precipitate was collected on a GF/A filter paper (2.5 cm square) and washed ten times with 2 ml of chilled 5% trichloroacetic acid followed by twice with 3 ml of ethanol. The radioactivity on the paper was counted in 10 ml of toluene-based scintillant with a Packard scintillation spectrometer.

Purification of DNA Ligase from Calf Thymus. The fresh thymuses from castrated oxen, which were obtained from Tokyo municipal slaughter house, were chopped into small pieces and homogenized in 3 volumes of 50 mM Tris-HCl pH 7.5/0.3 M KCl/10 mM mercaptoethanol/1 mM EDTA/0.5 mM phenylmethylsulfonyl fluoride with a home mixer (National MX-120) at the maximum speed for 1 min. The homogenate was centrifuged at 12000 x g for 30 min. From the supernatant solution obtained, DNA ligase was purified according to the following procedures: ammonium sulfate fractionation (35-60% saturation), calcium phosphate gel adsorption, column chromatographies on phosphocellulose, DEAE-cellulose, Blue-Sepharose CL-6B, Sephadex G-150 and DNA cellulose. DNA ligase was purified about 3000-fold with a specific activity of 4 x 10^4 units/mg protein over the supernatant solution. The purity (10-20%) of the enzyme preparation was judged by SDS-polyacrylamide gel electrophoresis [21] of [^{3}H]AMP-DNA ligase complex [22]. The purified enzyme preparation did not contain any detectable activities of the interfering enzymes which were assayed as described previously [12,23].

Determinations. Protein was determined by the method of Bradford [24] as well as by the method of Lowry et al.[25]. Other determinations were described previously [12].

RESULTS

As shown in Table 1, DNA ligase activity of calf thymus was stimulated by spermidine (0.5 mM) and spermine (50 μM) in the presence of 0.5 mM $MgCl_2$. The activation was completely dependent on the enzyme added. In the absence of Mg^{2+}, no enzymatic activity was detected by the addition of spermine (not shown) and spermidine, indicating that the polyamines do not substitute for Mg^{2+} in the enzyme reaction. Similar stimulation was observed by the addition of KCl (Table 1), NaCl and NH_4Cl (not shown) instead of the polyamines. The stimulation ratio of the enzyme activity by 0.5 mM spermidine was decreased

Table 1. Activation of DNA Ligase by Polyamines and KCl

Reaction mixture[a]	Ligase activity	
	pmol/10 min	%
Control system	0.69	100
+ 0.5 mM spermidine	1.51	218
- Enzyme, + 0.5 mM spermidine	<0.07	< 10
- $MgCl_2$, + 0.5 mM spermidine	<0.07	< 10
+ 50 μM spermine	1.58	229
+ 50 mM KCl	1.50	217
+ 0.5 mM spermidine, + 50 mM KCl	1.63	236

[a]The reaction mixture for control system was as described in Materials and Methods except for the presence of 0.5 mM $MgCl_2$.

gradually with increase in KCl concentration (see Table 1). The DNA ligase reaction proceeded linearly within at least 15 min under the conditions stated above.

The stimulation by spermidine was dependent on Mg^{2+} concentration in the reaction mixture (Fig. 1). The enzyme activity-Mg^{2+} concentration curve was slightly sigmoidal with the apparent Km value of 0.9 mM in the absence of spermidine. Spermidine (0.5 mM) not only lowered the Km value for Mg^{2+} to 0.5 mM but also elevated the V value along with the disappearance of the sigmoidicity. At 15 mM Mg^{2+} neither activation nor inhibition by 0.5 mM spermidine was observed. Similar results were obtained in the presence of KCl (50 mM) instead of spermidine. In the use of Mn^{2+} as a divalent cation (Fig. 2), DNA ligase activity was stimulated more than 3-fold by 0.5 mM spermidine even at the optimal concentration of $MnCl_2$ (0.2-0.5 mM). The apparent Km value of DNA ligase for ATP was about 3 mM regardless of the addition of 0.5 mM spermidine, whereas the V value was slightly stimulated. KCl and NaCl show no significant effect on the Km value for ATP and the V value as reported previously with rat liver DNA ligase [12].

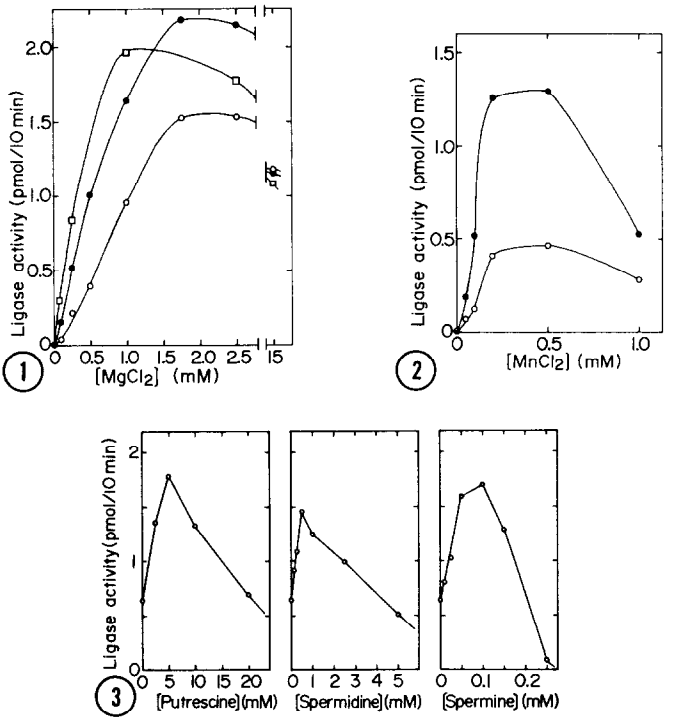

Fig. 1. Effect of Mg^{2+} concentration on the stimulation of DNA ligase activity by spermidine and KCl. The enzyme activity was determined as described in Materials and Methods except that Mg^{2+} concentration was varied and that 0.5 mM spermidine (●) or 50 mM KCl (□) was added to the control reaction mixture (○). Apparent Km values were obtained as Mg^{2+} concentrations giving half V value.

Fig. 2. Effect of Mn^{2+} concentration on the stimulation of DNA ligase activity by spermidine. Experimental conditions were as described in Materials and Methods except that the reaction mixture contained varying concentrations of Mn^{2+} instead of 15 mM Mg^{2+} with (●) or without (○) 0.5 mM spermidine

Fig. 3. Effects of increasing concentrations of polyamines on DNA ligase activity. Experimental conditions were as described in Materials and Methods except that the reaction mixture contained 0.5 mM $MgCl_2$ and varying concentrations of putrescine, spermidine and spermine as indicated in the abscissa.

Varying concentrations of putrescine, spermidine and spermine were tested for the effect on DNA ligase activity at the sub-optimal concentration of Mg^{2+} (0.5 mM). Putrescine stimulated the activity about 3-fold at the optimal concentration of 5 mM. Spermidine and spermine also stimulated the activity 2- to 3-fold at the optimal concentrations of 0.5 mM and 0.05-0.1 mM, respectively. At the higher concentrations of the polyamines, the activity was decreased gradually to complete inhibition (Fig. 3).

DISCUSSION

The activity of DNA ligase from calf thymus was stimulated by spermidine and spermine and their diamine precursor putrescine, all of which naturally occur in animal tissues. KCl and NaCl could replace the polyamines at the concentrations 10- to 1000-fold over those of polyamines.

The calf thymus enzyme seems to be identical with calf thymus DNA ligase I reported previously by Söderhäll and Lindahl [6,10]. In mammalian tissues, such as calf thymus and rat liver, two distinct DNA ligases had been found (reviewed in Ref. 26). Recently we suggested that DNA ligase of rat liver is localized in nucleus as a single species [20] which is probably identical with "cytoplasmic-type" enzyme previously reported [11,12]. During our purification procedures of DNA ligase from fresh calf thymus, DNA ligase II, the other species of DNA ligase which had been reported to be present in calf thymus and other tissues [6,10] could not be detected.

The "cytoplasmic-type" DNA ligase [12] partially purified from rat liver nuclei was also stimulated by the polyamines at the sub-optimal concentration of Mg^{2+} and at the optimal concentration of Mn^{2+} (unpublished results). As reported previously [12], the apparent Km value for Mg^{2+} of the cytoplasmic DNA ligase from rat liver was decreased by the addition of 50 mM KCl, whereas that for ATP was unaltered. In the presence of Mn^{2+} instead of Mg^{2+}, the V value was elevated more than 3-fold by the addition of 50 mM KCl. Therefore, DNA ligase of rat liver as well as the corresponding enzyme of calf thymus seems to be stimulated by polyamines and KCl in the identical fashion.

DNA ligase plays an essential role in DNA replication and repair in cooperation with DNA polymerases. DNA polymerase-α and -β from calf thymus have been found to be stimulated by polyamines at the sub-optimal concentration of Mg^{2+} and even at the optimal concentration of Mn^{2+} [27].

It is noteworthy that DNA ligase and DNA polymerases from calf thymus similarly respond to salts and polyamines in their reactions. Recent studies on the role of polyamines in mammalian DNA synthesis in vivo have suggested that the

accumulation of spermidine is required for DNA synthesis in regenerating rat liver [28,29]. It remains obscure whether polyamines are involved in the reactions in vivo of mammalian DNA ligase and DNA polymerases.

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