POLYNUCLEOTIDE LIGASE FROM RAT LIVER AFTER PARTIAL HEPATECTOMY

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SUMMARY: Polynucleotide ligase, which catalyzes the covalent joining of two segments of an interrupted strand in a DNA duplex, was extracted from rat liver. The activity is located in both nuclei and soluble fractions. Its optimal pH was 8.0. The enzyme requires ATP as a cofactor. Its activity was completely dependent on the presence of Mg^{2+}. The activities of these enzymes in regenerating rat liver were 4 times higher, in both fractions, than those in normal rat livers.

INTRODUCTION

Polynucleotide ligase catalyzes the synthesis of 3'-5'-phosphodiester bond, starting with a 5'-phosphoryl terminus and 3'-hydroxyterminus, of DNA chains which have been properly aligned in a double-helical structure. The cofactor for the T2, T4 and T7 bacteriophage induced enzyme is ATP (1-3), while that for the enzyme isolated from uninfected E. coli has been shown to be NAD (4-9). Recently, Lindahl and Edelman (10) have isolated this enzyme from rabbit bone marrow, and shown to require ATP as a cofactor. In the present communication we wish to describe some properties of a polynucleotide ligase from rat liver and the increased activity of this enzyme in regenerating rat liver.

MATERIALS AND METHODS

γ-^{32}P-ATP (sodium salt in 50 % aqueous ethanol, 1960 mc/mM) was the product of the Radiochemical Center (England). Calf thymus DNA, pancreatic DNase, alkaline phosphatase from E. coli and venom 5'-nucleotidase were from Sigma Chemical Company. Other enzymes were purchased from Worthington Biochemical Corp.

Partial hepatectomy refers to the removal of about 70 % of the liver (left

lateral and median lobes) (11). The preparation of rat liver nuclei and nuclear extract was employed by the same methods as described previously (12). The nuclei extract was dialyzed against 2 liters of 0.01 M Tris-HCl buffer (pH 7.7) for 2 hours at 2°C. Subcellular fraction was employed in the usual manner by centrifugation in medium A (13). Protein was determined by the method of Lowry (14) and DNA by the procedure of Burton (15).

The substrate of polynucleotide ligase, ^{32}P-labeled DNA was prepared by the same methods as previously described (12), using polynucleotide kinase from rat liver nuclear extracts.

The standard assay measures the conversion of 5'-^{32}P-phosphomonoesters in nicked DNA into a form which remains acid-insoluble after incubation with phosphatase. The incubation mixture (0.2 ml) contained 5 µg of 5'-^{32}P-phosphoryl nicked DNA (about 10,000 c.p.m), 10 µmoles of Tris-HCl buffer (pH 8.0), 2 µmoles of $MgCl_2$, 2 µmoles of mercaptoethanol, 0.04 µmoles of ATP and nuclear extract (about 10 µg of protein). After incubation at 37°C for 20 minutes, 0.1 ml of 1 M glycine buffer (pH 9.5) was added, and heated at 100°C for 10 minutes. The mixture was cooled in ice and 50 µg of alkaline phosphatase (1.5 unit) was added, and each reaction mixture was incubated for 20 minutes at 65°C. The mixture was cooled in ice, and 0.1 ml of 0.1 M sodium pyrophosphate was added, followed by 5 % trichloroacetic acid, the precipitate was collected on a glass filter. After extensive washes with trichloroacetic acid, ethanol, and ether, the samples were counted in a Packard Tri-carb liquid scintillation spectrometer.

RESULTS

With dialyzed nuclei extract and soluble fraction the activity in the standard assay was dependent on the addition of ATP and Mg^{2+} (Table I). A sulfhydryl compound was required for the optimum activity. In the absence of mercaptoethanol, 5×10^{-4} M p-chloromercuribenzoate inactivated 65 % of polynucleotide ligase, and this inactivation was reversed by adding 1.6×10^{-2} M mercaptoethanol, indicating that the enzyme has labile sulfhydryl groups.

Table I

Ligase Activity of Nuclear Extracts

Conditions of the standard assay were emoloyed.

Components	Activity
	mμmoles/mg of protein
Complete system	0.58
Minus Mg^{2+}	0.04
Minus enzyme	0.01
Minus mercaptoethanol	0.28
Minus ATP	0.02
Omit Mg^{2+}, add Mn^{2+}	0.02
Omit ATP, add NAD	<0.02
Minus mercaptoethanol	
Plus p-chloromercuribenzoate (5×10^{-4} M)	0.20
Plus p-chloromercuribenzoate (5×10^{-4} M) plus mercaptoethanol (1.6×10^{-2} M)	0.61
Single DNA	<0.01
Boiled enzyme (100°C, 3 minutes)	<0.01

Denaturation of DNA preparation containing internal ^{32}P-phosphomonoesters destroyed their ability to function as substrates.

The optimal pH for the reaction is 8.0 in Tris-HCl buffer. A time course for the polynucleotide ligase reaction is linear within 30 minutes. The reaction is directly proportional to enzyme concentration up to 20 μg of protein. The optimal Mg^{2+} concentration was about 0.015 M Mg^{2+}, which Mn^{2+} could not replace well Mg^{2+}. Half-maximal activity was found with 2×10^{-5} M ATP and NAD could not replace ATP.

Polynucleotide ligase can repair calf thymus DNA containing single strand

breaks. A sample of calf thymus DNA whose sedimentation rate in alkali has been reduced by incubation with pancreatic DNase was incubated with polynucleotide ligase and ATP. Not shown in the figure, after incubation the sedimentation coefficient in alkali increased from about 4 S to 6 - 7 S.

The cell fractions isolated were used to measure their content of polynucleotide ligase. Forty-five % of the activity was localized in the nuclear extracts, 15 % in microsome fraction, 40 % in the cytoplasma, and no activity was to be detected in the crude mitochondrial fraction.

Not shown in the table, the results were obtained by employing the same procedures as described previously (10) that this enzyme catalyzed the formation of 3'-5'-phosphodiester bond at the interrupted site.

The specific activities of polynucleotide ligase in nuclear extracts and 105,000 x g supernatant were estimated with normal and partially hepatectomized rats. The activities in the latter were 3 to 5 times higher, in both fractions than those in normal rat livers as shown in Table II.

Table II

Comparison of Polynucleotide Ligase Activity in Rat Liver from Normal and Partially Hepatectomized Rats (24 hours Post-Operatively)

Standard assay conditions were employed with nuclear extract (5 to 10 μg of protein) and 105,000 x g supernatant (40 to 80 μg of protein). Under the conditions of the assay of polynucleotide ligase in 105,000 x g supernatant, the reaction rate was proportional to the amount of enzyme (up to about 100 μg of protein).

Operative treatment	Cell fractions	
	Nuclear extract	105,000 x g supernatant
	Activity	
	mμmoles/mg of protein	
Normal	0.56 (0.68-0.51)	0.048 (0.062-0.044)
Partial hepatectomy	2.02 (2.29-1.30)	0.190 (0.230-0.162)

DISCUSSION

The polynucleotide ligase in higher organism was first described by Lindahl and Edelman (10) in rabbit bone marrow. They have shown that the enzyme required ATP as a cofactor, and ligase activity is highest in myeloid and lymphoid tissues, including bone marrow, spleen, and thymus, which undergo high levels of cell replication. The specific activities in crude extracts of intestine and liver were estimated to be 15- and 30-fold lower, respectively, than those in bone marrow. The results presented in this communication that the ligase is very active in nuclear extract in rat liver and required ATP as a cofactor.

Okazaki et al (16, 17) support the idea that relatively small polydeoxynucleotides are intermediates in the replication of both strands of bacterial and phage DNA, and they suggest also that the polynucleotides are subsequently joined to each other by polynucleotide ligase to the growing DNA strands. Recently, we have confirmed their observation in the tissues of higher organism that relatively low molecular weight polynucleotides are intermediates in replication of both strands of rat liver DNA in regenerating liver (18). In regenerating rat liver, the rise in nuclear DNA synthesis began about 12 - 16 hours after the operation and reached maximum at 19 - 23 hours. The present demonstration of relatively high ligase levels in rat liver at 23 hours after partial hepatectomy is consistent with their observation and suggests studies correlating increased DNA synthesis with higher ligase activity.

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