CHANGES IN POLYNUCLEOTIDE LIGASE DURING RAT LIVER REGENERATION

Kinji Tsukada

Drug Research Institute, Faculty of Pharmaceutical Science,
Toyama University, Toyama, Japan

Received February 14, 1974

SUMMARY: The specific activity of polynucleotide ligase in rat liver seems to begin to rise at 16 hours after partial hepatectomy (removal of 70% of the liver). The increases reach their maxima about 24 hours after operation, rising to at least 4 to 5 fold normal levels. Cycloheximide caused a decline in the increased activity of polynucleotide ligase. Since the specific activity of the ligase of normal rats is very little affected by cycloheximide, the possibility is considered that the newly formed enzyme is different from the one normally present in liver.

INTRODUCTION

The rise of DNA synthesis in the regenerating rat liver begins at about 14 hours after removal of 70% of the liver. It was reported that in DNA replication of regenerating rat liver relatively small polydeoxynucleotides are formed as intermediates (1, 2). Recently, we have shown that polynucleotide ligase is present in rat liver and that the activity of this enzyme is increased several fold at 24 hours after partial hepatectomy (3).

In this communication we wish to report the following results; (a) the increase in polynucleotide ligase activity after partial hepatectomy is related to the rise of DNA synthesis, (b) the newly formed enzyme after partial hepatectomy is different from the pre-existing one, having a much shorter half-life than the activity present in normal liver.

MATERIALS AND METHODS

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

Upjohn Company. Male albino rats (approx. 100g) were obtained locally and were given food and water *ad libitum*.

Partial hepatectomies were performed on rats according to the method of Higgins and Anderson (4). Rat liver nuclei and nuclear extracts were prepared by the methods described previously (5). The nuclei extract and soluble fraction (105,000 $\times g$ supernatant) were dialyzed against 0.01 M Tris-HCl buffer (pH 7.7) for 2 hours at 2°. Subcellular fractionation was carried out in the usual manner by centrifugation in Medium A (6).

The substrate of polynucleotide ligase, nicked 5'-phosphoryl-DNA-^{32}P, was prepared as previously described (5), with polynucleotide kinase from rat liver nuclear extracts. Polynucleotide ligase activity was assayed as previously described (3).

Protein was determined by the method of Lowry (7) and DNA by the procedure of Burton (8).

RESULTS AND DISCUSSION

The activity of polynucleotide ligase has been shown to reside in both nuclear extracts and soluble fractions, and to require ATP as a cofactor (3). The specific activity of polynucleotide ligase in the soluble fractions was measured as a function of time after partial hepatectomy (Fig. 1). As shown in figure, with soluble fractions, the rise of the activity of ligase began at about 16 hours with a lag phase and was maximal at about 24 hours after partial hepatectomy. The time curve of the response of ligase activity was similar to that of DNA synthesis measured with ^{3}H-thymidine incorporation after partial hepatectomy. The same results were obtained when the nuclear extracts were used, except that the values were about 10 times higher.

Cycloheximide has been reported to inhibit several cellular reactions in addition to protein and DNA formation (9-12). One mg of cycloheximide injected intraperitoneally inhibited liver protein synthesis by 95%, when assayed by the incorporation of ^{14}C-leucine into total liver protein during a 2 min pulse period at 10 min after the administration of cycloheximide.

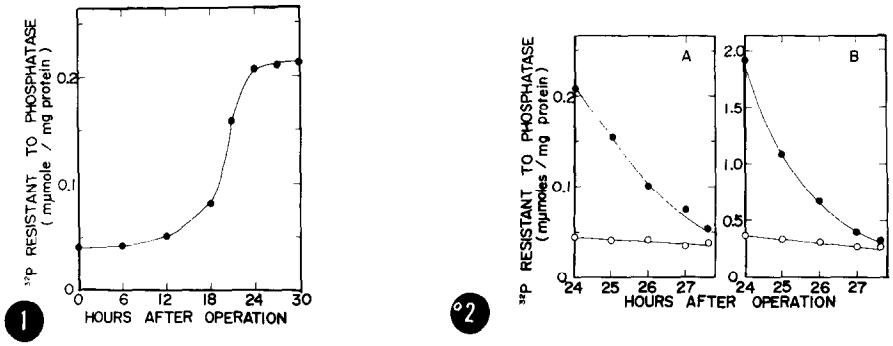

Fig. 1. Increase in the polynucleotide ligase activities of the soluble fractions from liver as a function of time after partial hepatectomy. The reaction mixture (0.2 ml) contained 5 μg of nicked 5'-phosphoryl-DNA-^{32}P (about 15,000 cpm), 10 μmoles of Tris-HCl buffer (pH 8.0), 2 μmoles of $MgCl_2$, 2 μmoles of mercaptoethanol, 0.04 μmole of ATP and soluble fraction (about 80 μg of protein). After incubation at 37° for 20 min, 0.1 ml of 1 M glycine buffer (pH 9.5) was added and heated at 100° for 10 min. The mixture was cooled in ice, and 5 μg of alkaline phosphatase (1.5 units) was added. Each 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. Each *point* shows the average of the results with 4 to 6 rats.

Fig. 2. Effect of cycloheximide on the specific activities of polynucleotide ligase in normal and partially hepatectomized rats. Cycloheximide (1 mg) in 0.15 M NaCl, 1 ml was given intraperitoneally to normal and partially hepatectomized rats at 24 hours after operation. At the times indicated, the liver samples (right lateral and caudate lobes) were removed. The specific activities of polynucleotide ligase were measured as described in Fig. 1. Each *point* shows the average of the results with 4 to 5 rats. ○, results obtained with normal rats; ●, with partially hepatectomized rats. A: soluble fraction, B: nuclear extracts.

Not shown in figure, evidence was obtained that DNA replication was markedly reduced at 5 min after administration of one mg of cycloheximide. We examined the effect of administration of cycloheximide *in vivo* on the polynucleotide ligase of rat liver. As shown in Fig. 2, the activity of polynucleotide ligase from partially hepatectomized rat liver decreased rapidly after treatment with cycloheximide, reaching the normal level at 3 to 4 hours in soluble fractions and 2 to 3 hours in nuclear extracts. There was little effect on the activity of polynucleotide ligase from normal rat. A reasonable explanation for this result would seem to be that the ligase formed after hepatectomy

was or contained a different species of protein from the activity normally present in liver cells.

Pedrini *et al* (13) reported that in human lymphocytes phytohemoagglutinin causes the rise of the activity of polynucleotide ligase after 4 or 5 day's lag after treatment and this increase is delayed by one day with respect to the rise of DNA synthesis. Ligase is implicated in the current models of DNA replication and its involvement in that process has been satisfactorily proven in phage T_4 (14) and in *E. coli* (15). In kidney tumors in rats, we have shown that the rate of thymidine incorporation into DNA is correlated with the ligase activity in tumors (16).

In hepatomas, thymidine incorporation into DNA and growth rate showed a good correlation (17, 18). The ligase activity in hepatoma induced by 2-fluorenylacetamide is several times greater than that in normal rat liver (19). These results suggest that ligase activity is correlated with increased DNA synthesis.

ACKNOWLEDGEMENT: This investigation has been supported in part by the Scientific Research Fund for Cancer from the Ministry of Education, Japan.

REFERENCES

1. Tsukada, K., Moriyama, T., Lynch, W. E., and Lieberman, I., Nature, *220*, 162 (1968).
2. Berger, H., and Irvin, J. L., Proc. Natl. Acad. Sci., U. S. A., *65*, 152 (1970).
3. Tsukada, K., and Ichimura, M., Biochem. Biophys. Res. Commun., *42*, 1156 (1971).
4. Higgins, G. M., and Anderson, R. M., Arch. Pathol., *12*, 186 (1931).
5. Ichimura, M., and Tsukada, K., J. Biochem., (Tokyo) *69*, 823 (1971).
6. Hoagland, M. B., Stephenson, M. L., Scott, J. F., Hecht, L. I., and Zamecnik, P. C., J. Biol. Chem., *231*, 241 (1958).
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem., *193*, 265 (1951).
8. Burton, K., Biochem. J., *61*, 473 (1955).
9. Gross, C., Biochem. Biophys. Acta, *166*, 40 (1968).
10. Greenberger, N. L., and Ruppert, R. D., Science, *153*, 315 (1966).
11. MacDonald, I. R., and Ellis, R. J., Nature, *222*, 791 (1969).
12. Brown, R. F., Umeda, T., Takai, S., and Lieberman, I., Biochem. Biophys. Acta, *209*, 49 (1970).
13. Pedrini, A. M., Zuzzo, F., Ciarrocchi, G., Palpra, L., and Falaschi, A., Biochem. Biophys. Res. Commun., *47*, 1221 (1972).

14. Fareed, G. C., and Richardson, C. C., Proc. Natl. Acad. Sci., U. S. A., *58*, 665 (1967).
15. Modrich, P., and Lehman, I. R., Proc. Natl. Acad. Sci., U. S. A., *68*, 1002 (1971).
16. Tsukada, K., and Ito, N., J. Biochem., (Tokyo) *72*, 1299 (1972).
17. Lea, M. A., Morris, H. P., and Weber, G., Cancer Res., *26*, 465 (1966).
18. Lea, M. A., Morris, H. P., and Weber, G., Cancer Res., *32*, 886 (1972).
19. Tsukada, K., Hokari, S., Hayasaki, N., and Ito, N., Cancer Res., *32*, 886 (1972).