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Regenerating rat liver DNA polymerases : dissimilitude or relationship between nuclear and cytoplasmic enzymes ?

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ABSTRACT

The possible relationship between the nuclear and cytoplasmic DNA polymerases of regenerating rat liver was studied by sucrose gradient analysis, salt dissociation, and with specific inhibitors. After aqueous subcellular fractionation and removal of the nuclear membranes, three species of DNA-dependent DNA polymerases were characterized : 1) a DNA polymerase-β in the nuclei. 2) a DNA polymerase-α in the cytosol which was not dissociated at high salt concentrations ; and 3) an intermediate form in the cytosol and in the Triton wash containing the nuclear membranes. The latter form behaved like DNA polymerase-α et low salt concentrations but was dissociated at high salt concentrations to a low molecular weight species with properties like DNA polymerase-β (resistance to inhibition by N-ethylmaleimide, heparin and KCl). In vitro reassociation experiments suggest that this intermediate form corresponds to the association of DNA polymerase-β with a membrane component or cytoplasmic protein(s) which appear(s) in regenerating rat liver.

INTRODUCTION

Mammalian cells have been found to contain distinct molecular species of DNA polymerases. One of these, DNA polymerase-α , has a molecular weight in the range of 100,000 to 200,000 daltons. Although generally found in the cytoplasm (1-4), it has been reported in the nucleus (2,3,4). DNA polymerase-β has a low molecular weight of about 45,000 daltons and is found predominantly in the nucleus (5,6). Animal cells also contain an R-dependent DNA polymerase, i.e. DNA polymerase-γ (7), and a mitochondrial DNA polymerase (8).

Some experiments have indicated that DNA synthesis was correlated with an increased activity of the DNA polymerase-α (9,10), whereas the β -polymerase activity has been reported to be relatively constant during all phases of the cell cycle (7,11).

The significance of the presence of DNA polymerase-α in the cytoplasm is not thoroughly understood. It may be an artefact caused by leakage of the enzyme from the nucleus during cell fractionation in aqueous media (12-14). Another possibility is that α -polymerase may be

synthetized in the cytoplasm and transported to the nucleus during the S phase.

Immunological and structural relationships between DNA polymerases α and β have been suggested (15-17). But subsequent immunological studies (18,19) failed to confirm the earlier observation (15). The tendancy of "cytoplasmic" and nuclear enzymes to undergo ionic strength-dependent aggregation reactions also showed that these two DNA polymerase species could not be clearly distinguished only on the basis of size criteria (1,20).

We have previously described the purification and the enzymatic properties of a regenerating rat liver DNA polymerase-α which sediments at 7.4 S at high ionic strength and which cannot be dissociated to a low molecular weight species (21). Using N-ethylmaleimide,heparin or high KCl concentrations as specific inhibitors of DNA polymerase-α, we have studied the intracellular localization of this enzyme during liver regeneration in an attempt to detect its possible transport to the nucleus, its concentration at the nuclear membrane level and its possible structural modifications in the nucleus.

MATERIALS AND METHODS

Preparation of nuclear and cytosol enzyme extracts. The cytosol was prepared using a method previously described (22). Samples of liver, removed at different times after hepatectomy, were disrupted with a Potter homogenizer in 3 vol of Buffer A (50 mM Tris-HCl (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$ and 0.25 M sucrose) and centrifuged 2 h at 105,000 x g.

The nuclei were isolated using a modification of Chauveau's method (23).Liver tissue was homogenized in 15 vol of Buffer B (50 mM Tris-HCl (pH 7.5), 25 mM KCl, 2 mM $MgCl_2$, 3 mM $CaCl_2$ and 2.2 M sucrose) and centrifuged at 25,000 revs/min for 60 min in a Spinco n°30 rotor. The nuclear pellets were resuspended in Buffer A containing 0.5% TritonX-100 to remove the nuclear membranes (24). The suspension was allowed to stand for 15 min at 4°, and the nuclei were collected by centrifugation at 600 x g for 10 min in a Sorvall centrifuge. The nuclei were then washed 3 times in Buffer A, without Triton X-100. The final nuclear pellet was resuspended in 0.2 M potassium phosphate buffer (pH 7.5), containing 1 mM 2-mercaptoethanol, and extracted for 2 h at 4° with occasional gentle stirring. The nuclear extract was then clarified at 105,000 x g for 2 h, and stored at - 80°. The purity of the nuclei and the absence of nuclear membranes after the Triton treatment were controlled by electron microscopy.

Sucrose gradient centrifugation. Preformed 5 to 20% sucrose gradients were prepared in Buffer C (50 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM 2-mercaptoethanol) containing various concentrations of KCl.

An aliquot of the cytoplasmic or nuclear extracts dialyzed against the same buffer was loaded onto a preformed gradient and centrifuged for 15 h at 40,000 revs/min at 4° in a Spinco SW50 L rotor unless otherwise stated in the legends to the figures. The gradients were fractionated into about 30 equal fractions, and aliquots (20 µl) were assayed for DNA polymerase. Yeast alcohol dehydrogenase (7.4 S) and bovine liver catalase (11.3 S) were used as markers. Sedimentation coefficients were determined according to the procedure of Martin and Ames (25).

DNA polymerase assays. The standard reaction mixture (50 µl) contained 50 mM Tris-HCl at pH 7.6 or 8.6, 3 mM $MgCl_2$, 100 mM 2-mercaptoethanol, 22 µM poly(dC).$(dG)_{12-18}$, 100 µM [^{3}H] dGTP (1.2 µCi), 20 µl of enzyme fraction, 50 µg of bovine serum albumin and KCl at concentrations as indicated in the legends. After a 60-min incubation at 37°, 40 µl of the reaction mixture were transferred to a Whatman GF/C glass fiber disc, processed according to the procedure previously described (22) and the radioactivity of the acid insoluble material was determined by liquid scintillation counting. The use of poly(dC).$(dG)_{12-18}$ as template at pH 8.6 in the presence of Mg^{++} allowed the detection of DNA polymerase-α and β under the same conditions. The results were expressed in nmoles of dGMP incorporated per fraction in 1 hour at 37° and have been corrected to correspond to 1 g of tissue.

RESULTS

DNA polymerase activities in the cell fractions. The DNA polymerase activities in the cytoplasmic and nuclear fractions of normal and regenerating rat liver were investigated by analysis of solubilized enzymes on sucrose gradients under conditions of ionic strength that favor a good separation of α and β -polymerases.

The 6.8 S and 3.4 S DNA polymerases found in the cytoplasmic fraction of regenerating rat liver are shown in Figure 1A. In our experiments, the low molecular weight species was the only activity detected in the cytosol of control livers as Holmes, Hesslewood and Johnston (26) found for adult animals (Fig.1C).

In the regenerating rat liver nuclei, washed with a 0.5% solution of Triton X-100 to remove the nuclear membranes, only a 3.4 S DNA polyme-

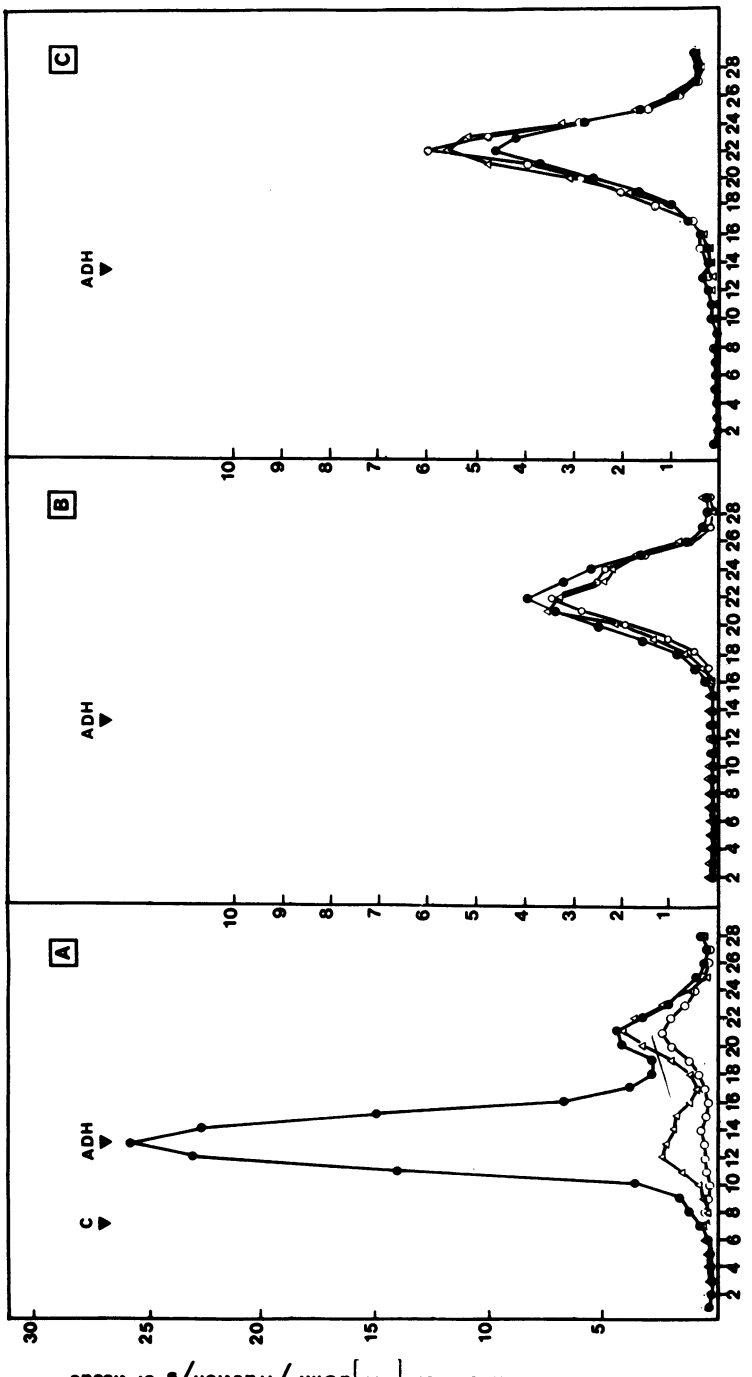

Figure 1 : DNA polymerase activities of the cytosol and nuclear fractions of normal and regenerating rat liver : 300 µl of cytosol or nuclear extracts were dialyzed against Buffer C containing 250 mM KCl, loaded on a preformed sucrose gradient prepared in the same buffer and centrifuged as described under "Materials and Methods". Sedimentation was to the left. The enzyme activities were assayed with poly(dC).(dG) 12-18 as template, at pH 7.6 for A and C, and at pH 8.6 for B. A) Cytosol extract of 40-hour regenerating rat liver. B) Nuclear extracts of the same livers. C) Cytosol extract of normal adult rat livers. The DNA polymerase activities were tested in the presence of 100 mM 2-mercaptoethanol (●—●), or in the presence of 1 mM N-ethylmaleimide (Δ—Δ), or in the presence of 0,15 µg of heparin (Choay, Paris France) (O—O). The KCl concentration during the polymerase assay was 100 mM KCl.

rase was detected (Fig. 1B) and this activity remained relatively constant after hepatectomy.

Like DNA polymerase-β(1,20), the 3.4 S enzymes (nuclear or cytoplasmic) were not inhibited by N-ethylmaleimide or by heparin, whereas the high molecular weight enzyme that appeared in the cytoplasmic fraction after hepatectomy was inhibited by these two compounds, as previously demonstrated for highly purified DNA polymerase-α(21)(Fig. 1A,B and C).

Since we had not detected a DNA polymerase-α in the nuclei of regenerating rat liver under our experimental conditions, we performed a gradient analysis of the Triton wash containing the nuclear membranes. A sharp and transient increase of a high molecular weight species appeared in this fraction at 24 h or 40 h after hepatectomy depending on the experiments, but always during DNA synthesis (Fig. 2). We have tried to characterize this DNA polymerase activity.

Comparative study of high molecular weight DNA polymerases detected in the cytosol and in the Triton wash of the nuclei from regenerating rat liver

We have studied the influence of salt concentration on these two activities in an attempt to detect possible ionic strength-dependent aggregation or dissociation reactions.

When a 40-hour regenerating rat liver cytoplasmic extract was dialyzed against Buffer C containing 10 mM KCl and loaded on a sucrose gradient prepared in the same buffer, a major peak of DNA polymerase activity was detected sedimenting at about 11 S; very low DNA polymerase activity was detected at 3.4 S, even in the presence of 100 mM KCl in the reaction mixture (optimal concentration of salt for low molecular weight DNA polymerase) (Fig. 3-1).

When the same supernatant was dialyzed against Buffer C containing 350 mM KCl and loaded on a sucrose gradient prepared in the same buffer, the DNA polymerase activity was separated into two peaks sedimenting at 7.4 S (A) and 3.7 S (B) (Fig. 3-2). Peak A had the enzymatic properties of DNA polymerase-α and Peak B had the properties of DNA polymerase-β, that is the same optimal KCl concentrations (Fig. 3-2) and the same sensitivities to N-ethymaleimide and heparin (Table 1). The formation of two peaks of DNA polymerase from the cytoplasmic fraction is an important point because the highly purified DNA polymerase-α was not dissociated to the 3.4 S species at high ionic strength (21). After dialysis at low ionic strength, Peak A again sedimented at 10 - 11 S like purified DNA

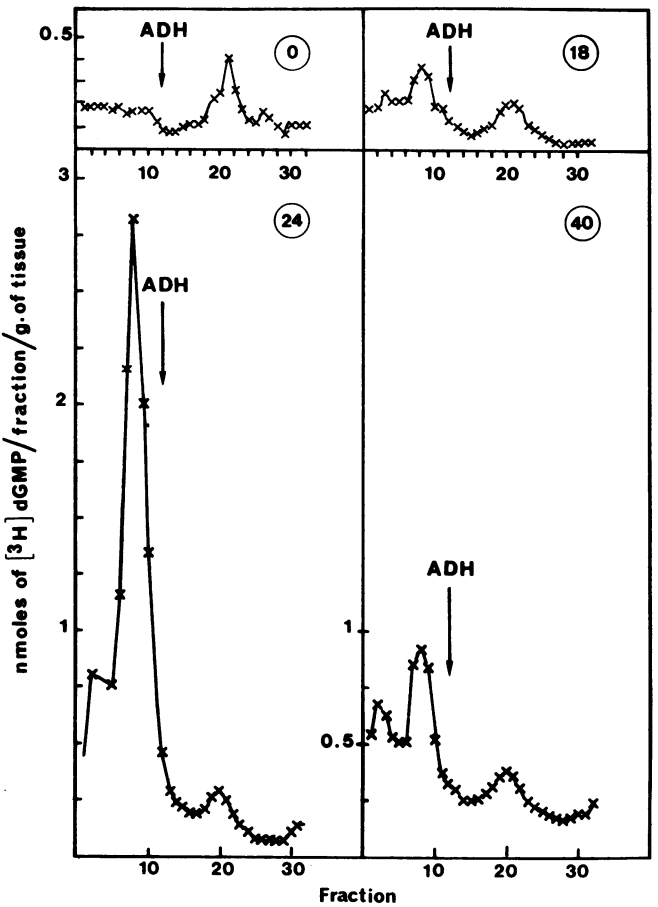

Figure 2 : DNA polymerase activities detected in the Triton wash of nuclei purified from rat liver removed at different times after partial hepatectomy : 200 µl of 5% Triton X-100 solution, which had been used to remove the membranes of purified nuclei, were dialyzed overnight against Buffer C containing 100 mM KCl, loaded on a preformed sucrose gradient prepared in the same buffer and centrifuged 16 h at 40.000 revs/min in an SW50 L rotor. DNA polymerase activity was tested with poly(dC).(dG)12-18 as template at pH 8.6 and in the presence of 40 mM KCl.

The arrow marked ADH indicates the sedimentation position of alcohol dehydrogenase (7.4 S). In this experiment the Triton X-100 modified the flow of the light fractions and the calculation of the sedimentation coefficient could not be precisely determined as compared to the markers.

polymerase-α . In contrast, repeated attempts to reaggregate Peak B by dialysis failed. The cytoplasmic fraction therefore contained as described by Hecht (27) two species of large DNA polymerase activity, only one of which is converted by salt to a 3.4 S form.

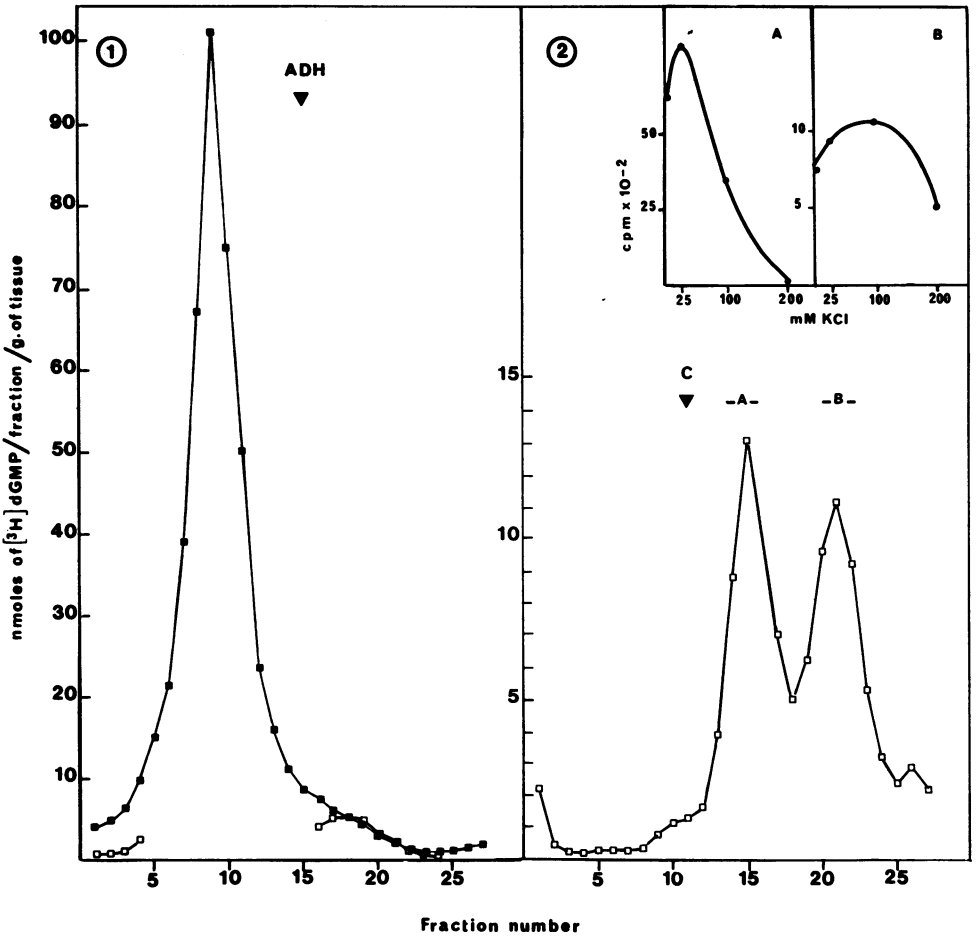

Figure 3 : Partial salt dissociation of the heterogeneous high molecular weight DNA polymerase present in the cytosol of regenerating rat liver : 200 µl of cytosol fraction of 40-hour regenerating rat livers were dialyzed overnight against Buffer C containing 10 mM KCl (1) or 350 mM KCl (2), loaded on a preformed sucrose gradient prepared in the same buffers, and centrifuged 15 h at 37.000 revs/min in an SW50 L rotor. DNA polymerase activity was tested in the presence of poly(dC).(dG)12-18 at pH 8.6. 1) DNA polymerase was assayed in the presence of 4 mM KCl (■—■) or in the presence of 100 mM KCl (□—□), 2) DNA polymerase was assayed in the presence of 140 mM KCl (□—□). In the corner, the DNA polymerase activities of peaks A and B as a function of the KCl concentration in the incubation medium.

The arrows marked ADH and C indicate the sedimentation position of alcohol dehydrogenase (7.4 S) and catalase (11.3 S).

On the other hand, the DNA polymerase activity detected in the Triton wash containing the nuclear membranes sedimented at 10-11 S in the presence of 10 mM KCl and completely dissociated to a low molecular

TABLE 1

EFFECTS OF VARIOUS COMPOUNDS ON RAT LIVER DNA POLYMERASES

Enzymes	Control	+ N-ethyl-maleimide	+ heparin	+ anti-DNA pol.-β IgG	Optimal KCl concentration	
		% Activity of controls			4-10 mM	100-150 mM
RR liver:						
cytosol (7.4 S)	100	10*	1 to 2		+	
nuclei (3.4 S)	100	95	95			+
N rat liver						
cytosol (3.4 S)	100	⩾ 100	⩾ 100			+
nuclei (3.4 S)	100	95	95			+
RR liver cytosol treated by 350 mM KCl						
peak 7.4 S (A)	100	46*	0		+	
peak 3. 7 S (B)	100	100	100			+
Triton wash (RR rat liver nuclei)						
heavy peak	100	13*	1	130	+	
light peak	100	95	100	45		+
RR liver cytosol + N rat liver nuclei extracts						
heavy peak	100	20*	0			
light peak	100	100	80			

Standard assay conditions were used with the additions indicated in the Table. In experiments testing the effect of N-ethylmaleimide, 2-mercaptoethanol was omitted from the incubation mixture. Heparin, anti-DNA polymerase-β IgG, and N-ethymaleimide were tested in the presence of the optimal KCl concentration for the enzyme studied. Abbreviations : N rat liver : normal adult rat liver, RR liver : regenerating rat liver removed 40 h after hepatectomy.

The activities of the high molecular weight DNA polymerases* detected in the sucrose gradient fractions of the crude extracts were not completely inhibited by N-ethylmaleimide as was the highly purified DNA polymerase-α . This could be due either to partial overlapping of the heavy and light peaks (see Fig.3) or to incomplete dissociation of the polymeric form of DNA polymerase-β (Fig. 1).

weight species at high ionic strenght (Fig. 4 A). The dissociation involved a modification of the properties of this enzyme fraction which behaved like DNA polymerase-α at low salt concentrations (it was inhibited by N-ethylmaleimide, heparin and KCl) and which acquired at high ionic strength all the characteristics of DNA polymerase-β (low molecular weight and resistance to inhibition by N-ethylmaleimide, heparin and high salt concentrations)(Fig. 4B and Table 1). In addition, antibodies prepared against the DNA polymerase-β of chick embryos* inhibited 60% of the

* The anti-DNA polymerase-β immunoglobulins were kindly provided by Dr. G. Brun. These antibodies inhibit hamster DNA polymerase-β , whereas no significant neutralization of hamster DNA-polymerase-α has been observed in the presence of antibodies prepared against chick embryo DNA polymerase-α . Cross reactions have not been obtained between the high molecular weight DNA polymerases from avian and mammalian species (19).

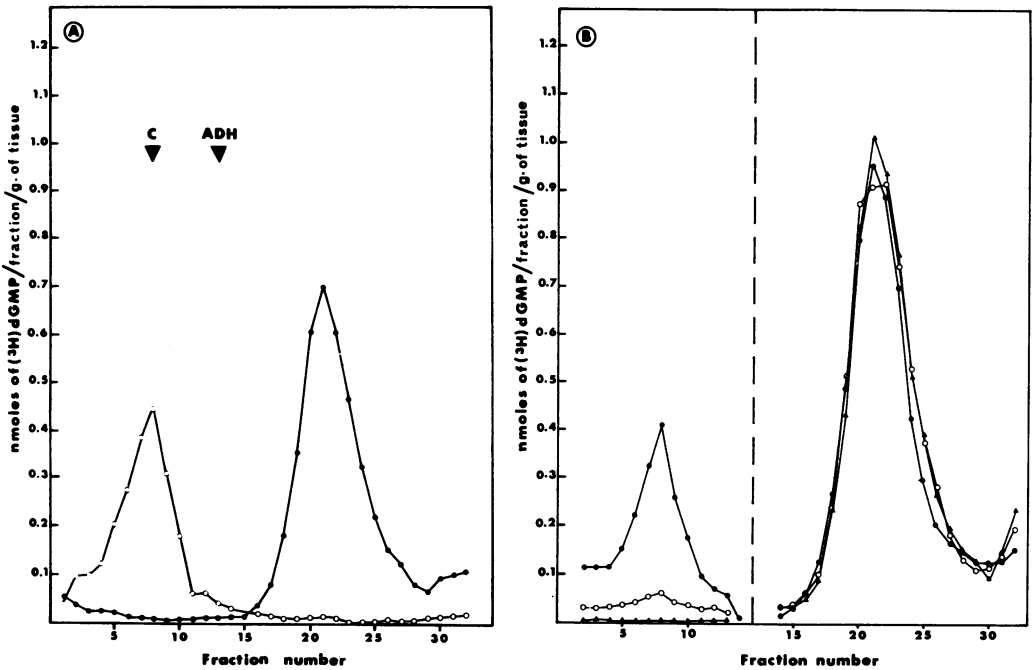

Figure 4 A: Complete salt dissociation of the high molecular weight DNA polymerase present in the Triton wash of 40-hour regenerating rat liver nuclei :The sucrose gradient analysis of the DNA polymerase activity present in 300 µl of the 0.5% Triton X-100 buffer used to remove the membranes of purified nuclei was performed as described in Fig. 3 ; sedimentation was performed in a sucrose gradient containing 10 mM KCl (O—O) or 350 mM KCl (●—●). The KCl concentration during polymerase assay was 4 mM (O—O) or 140 mM (●—●).

Figure 4 B : Modification of the properties of the DNA polymerase present in the Triton wash of regenerating rat liver nuclei after salt dissociation. The sedimentation was performed in gradient containing 10 mM KCl (left) or 350 mM KCl (right). DNA polymerase was tested in the presence of 100 mM 2-mercaptoethanol (●—●), 1 mM N-ethylmaleimide (O—O) or 0,15 µg of heparin (▲—▲) at 4 mM KCl (left) or at 140 mM KCl (right).

The arrows marked ADH and C indicate the sedimentation positions of alcohol dehydrogenase (7.4 S) and catalase (11. 3 S).

As indicated in Fig. 2, the exact sedimentation coefficients could not be determined in the presence of Triton X-100.

activity of this enzyme in its low molecular weight form (Fig. 5), whereas they were inactive with the 10-11 S complex formed at low salt concentrations. As we have indicated for the cytosol, the low molecular weight DNA polymerase obtained by salt dissociation could not be reaggregated after dialysis.

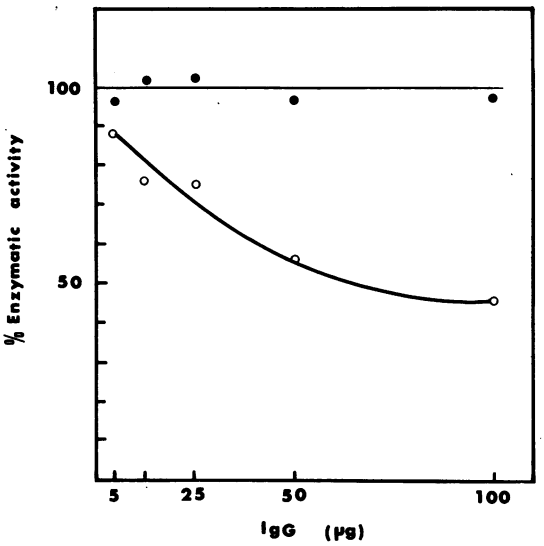

Figure 5 : Neutralization effect of anti DNA polymerase-β IgG on DNA polymerase of Triton wash. Standard neutralization assays were performed as described by Brun et al. (15) and contained 30 µl of DNA polymerase dissociated in the presence of 350 mM KCl (fraction 20,21,22 of Fig. 4). One hundred per cent activity was determined with immunoglobulins purified from normal rabbit serum, and corresponded to approximately 20 000 cpm of dGMP incorporated in 1 hour by 30 µl of enzyme fraction (●–●) ; activity measured in the presence of anti DNA polymerase-β IgG (O–O). Before salt dissociation, the DNA polymerase of Triton wash was not inhibited but stimulated by anti DNA polymerase-β IgG and by normal rabbit serum immunoglobulins (Table 1).

Are salt-dissociable DNA polymerases aggregates of DNA polymerase-β or an association of DNA polymerase-β with other factor(s) ?

Wang et al. (20) have shown that the nuclear DNA polymerase-β from KB cells formed at low ionic strength large aggregates of a size comparable to that of DNA polymerase-α , but several of their results suggested that the aggregation of DNA polymerase-β was not due simply to the self-association of β -polymerase monomers but rather required the participation of some additional factor(s) . We have examined this question in our system.

In our experiments, low molecular weight enzyme reaggregation was obtained only by dialysis of the crude extracts (cytosol or Triton wash) and never by dialysis of the 3.4 S peaks. This would indicate that at least two components of different size were dissociated by the salt and separated during ultracentrifugation.

Moreover, the nuclear DNA polymerase of normal adult rat liver did not aggregate at low ionic strength (Fig. 6), whereas a partial aggregation of the nuclear enzyme of regenerating rat liver was obtained. It is therefore difficult to consider that the salt dissociable activities detected in regenerating rat liver cytosol and Triton wash was only a polymeric form of the DNA polymerase-β . These activities may represent,

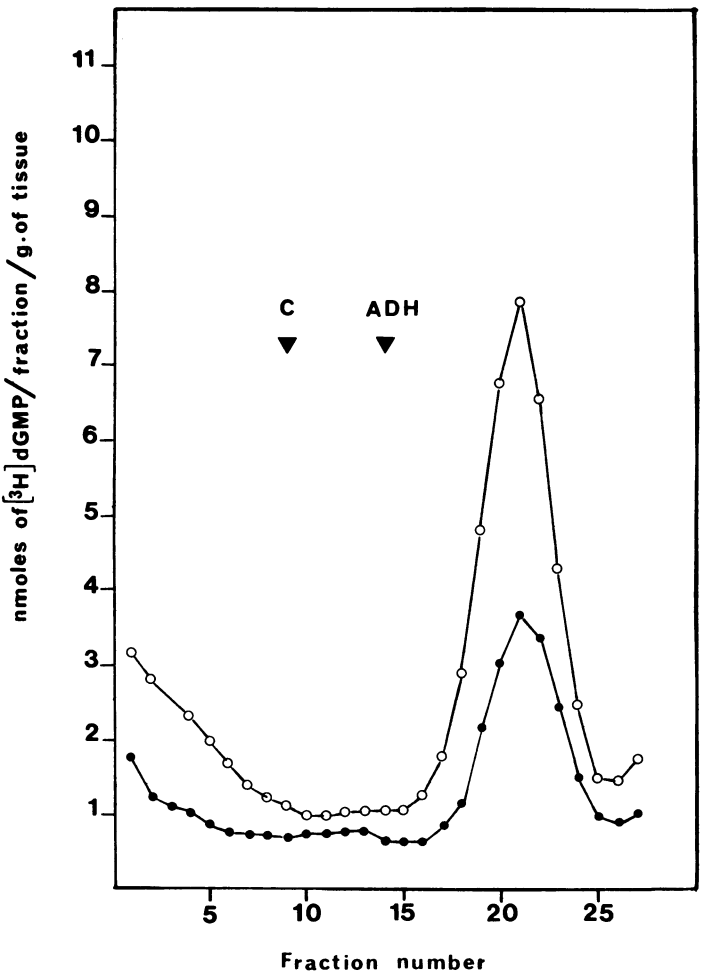

Figure 6 : Absence of aggregation of nuclear enzyme at low ionic strength: 200 µl of nuclear extract from normal rat liver were dialyzed against Buffer C containing 10 mM KCl, loaded on a preformed sucrose gradient prepared in the same buffer, and centrifuged 15 h at 37 000 revs/min in an SW50 L rotor. DNA polymerase activity was tested at 4 mM KCl (●—●) or at 100 mM KCl (○—○).

The arrows marked ADH and C indicate the sedimentation positions of alcohol dehydrogenase (7.4 S) and catalase (11.3 S).

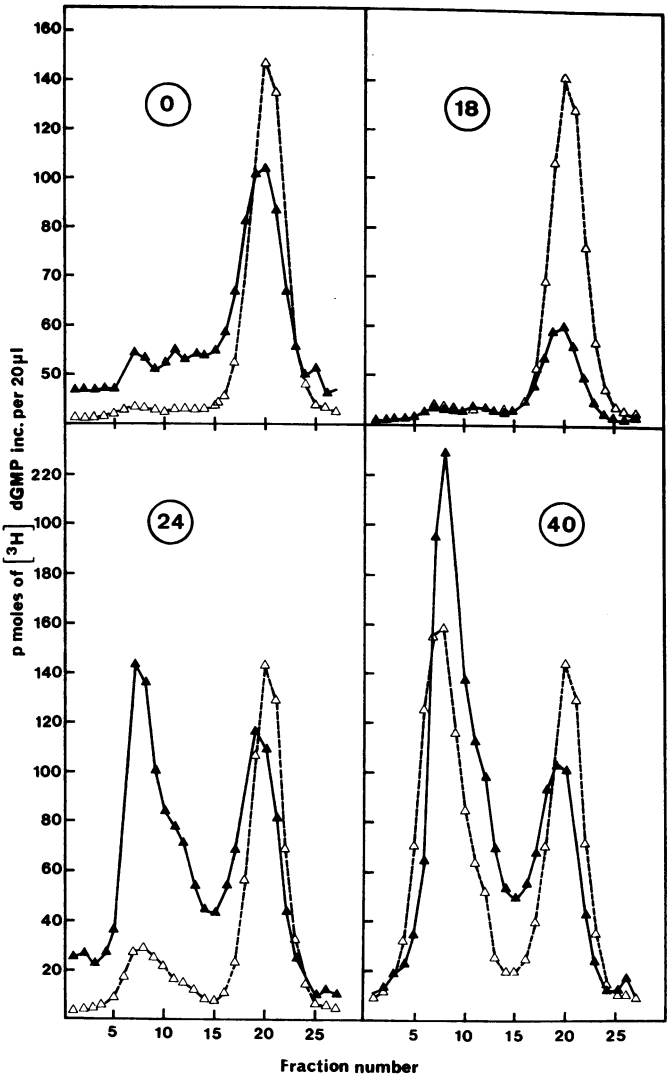

Figure 7 : Reassociation of nuclear DNA polymerase with cytoplasmic protein(s) of regenerating rat liver : 100 µl of nuclear extract of normal rat livers were dialyzed with 100 µl of cytosol extract of normal (o) or regenerating rat liver removed 18,24 or 40 hours after hepatectomy ((18) , (24) , (40))against Buffer C containing 100 mM KCl. Then, the mixtures were loaded on preformed sucrose gradients prepared in the same buffer. DNA polymerase was tested at pH 8.6 and in the presence of 40 mM KCl. The solid triangles (▲—▲) represent the experimental data obtained when the dialyzed mixture was centrifuged. The light triangles (△—△) indicate the expected activity of theoretical mixtures, calculated from the data obtained when the nuclear and cytoplasmic extracts were centrifuged separetely. Activities were expressed as pmoles of radioactive nucleotide incorporated per 20 µl of gradient fraction in 1 hour at 37°.

as suggested by recent work (28,29), the interaction of the 3.4 S sedimenting DNA polymerase with other factors.

In fact, when we dialyzed at low ionic strength a mixture of a nuclear extract of normal rat liver with a cytoplasmic supernatant of regenerating rat liver removed at different times after hepatectomy, heterogeneous high molecular weight species appeared in the sucrose gradient especially when a 24-hour supernatant was used (Fig. 7). This phenomenon did not occur when a cytoplasmic supernatant of control liver was used or when the regenerating rat liver cytosol was previously boiled, or when the extracts were treated with N-ethylmaleimide. This resulting high molecular weight DNA polymerase activity was inhibited by heparin and N-ethylmaleimide as was the activity found in the Triton wash before dissociation by salt (Table 1).

DISCUSSION

After the membranes of purified nuclei were removed by Triton, we were able to identify three types of DNA-dependent DNA polymerases in regenerating rat liver:

1) A nuclear DNA polymerase having the characteristics of DNA polymerase-β.

2) A cytoplasmic DNA polymerase having the characteristics of DNA polymerase-α and capable of passing from a 10-11 S form to a 7.4 S form at high salt concentrations as was shown for a highly purified DNA polymerase-α (21), however, it was not dissociable to 3.4 S DNA polymerase.

3) A DNA polymerase found essentially at the level of the nuclear membranes and the cytosol which sedimented at 10-11 S at low ionic strength. This polymerase had enzymatic properties like DNA polymerase-α: it was inhibited by N-ethylmaleimide, heparin and high salt concentrations. This enzyme was entirely dissociated by salts to low molecular weight species and had in this form, all the properties of DNA polymerase-β; in particular, it was inhibited by monospecific antibodies directed against the DNA polymerase-β of chick embryos. This intermediate form appeared at the onset of DNA synthesis.

After fractionation of the cells in aqueous media, we were unable to detect DNA polymerase-α in the nuclei or in the nuclear membranes of regenerating rat liver, in contrast with the results described by Lynch et al. (12) and Baril et al. (4) but in accord with those of Chang and

Bollum (5). This could be in part due to the composition of our homogenization medium (50 mM Tris and 25 mM KCl).

As found by Hecht (27) and Wang et al. (20), we were able to detect a heavy form entirely dissociable to β-polymerase. In our experiments this structural change resulted in a change in its properties, whereas for Wang et al., the aggregate retained the distinctive features of DNA polymerase-β monomer. We have also shown that this intermediate form corresponded to the association of DNA polymerase-β with a membrane component(s) or protein(s) which appear(s) in regenerating rat liver. This phenomenon could play an important role in the regulation of the DNA replication process and could well explain the contradictory results published during the past few years. The elimination of the membranes of the nuclei is not always realized or controlled, moreover the possibility of detecting a high molecular weight DNA polymerase species in the nuclei may vary according to the tissues or the cells studied since a membrane component(s) or protein(s) found to associate with the DNA polymerase increase(s) in actively dividing cells.

During liver regeneration, the activities of DNA polymerase-α and an intermediate form increase at the onset of DNA synthesis. But heir exact role has not yet been determined. We believe that the replication complex is a polymeric structure that is most likely destroyed during the preparation of cellular extracts. It probably consits of one or several DNA polymerases associated with other proteins also implicated in replication. Our future studies will concern the proteins that can associate with the DNA polymerase in the cytosol and the Triton wash of regenerating rat liver to determine their role in the DNA replication process.

ACKNOWLEDGMENTS

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REFERENCES

1. Bollum, F.J. (1975) Progress in Nucleic Acid Research 15, 109-144.
2. Weissbach, A., Schlabach, A., Fridlender, B. and Bolden, A. (1971) Nature, 231, 167-170.
3. Sedwick, W.D., Wang, T.S.F., and Korn, D. (1972) J. Biol. Chem. 247, 5026-5033.

4. Baril, E.F., Jenkins, M.D., Brown, O.E., Laszlo, F. and Morris, H.P. (1973) Cancer Research, 33, 1187-1193.
5. Chang, L.M.S., and Bollum, F.J. (1971) J. Biol. Chem. 246, 5835-5837.
6. Chang, L.M.S. (1973) J. Biol. Chem. 248, 3789-3793.
7. Spadari, S. and Weissbach, A. (1974) J. Biol. Chem. 249, 5809-5815.
8. Fry, M. and Weissbach, A. (1973) Biochemistry 12, 3602-3608.
9. Chang, L.M.S. , and Bollum, F.J. (1972) J. Biol. Chem. 247, 7948-7950.
10. Chang, L.M.S., Brown, M. and Bollum, F.J. (1973) J. Mol. Biol. 74, 1-8.
11. Chiu, R.W., and Baril, E.F. (1975) J. Biol. Chem. 250, 7951-7957.
12. Lynch, W.E., Surrey, S., and Lieberman, I. (1975) J. Biol. Chem. 250, 8179-8183.
13. Foster, D.N., and Gurney, T. (1973) J. Cell Biol. 59, 103 A .
14. Herrick, G., Spear, B.B., and Veomett, G. (1976) Proc. Natl. Acad. Sci. U.S.A. in press.
15. Chang, L.M.S. and Bollum, F.J. (1972) Science, 175, 1116-1117.
16. Hecht, N.B. (1973) Biochim. Biophys. Acta, 312, 471-483.
17. Lazarus, L.H. and Kitron, N. (1973) J. Mol. Biol. 81, 529-534.
18. Spadari, S., Muller, R. , and Weissbach, A. (1974) J. Biol. Chem. 249, 2991-2995.
19. Brun, G., Assairi, L.M. and Chapeville, F. (1975) J. Biol. Chem. 250 7320-7323.
20. Wang, T.S.F., Sedwick, W.D., and Korn, D. (1975) J. Biol. Chem. 250, 7040-7044.
21. Fichot, O., Pascal, M., and de Recondo, A.M. , submitted for publication.
22. de Recondo, A.M., Lepesant, J.A., Fichot, O., Grasset, L., Rossignol, M. and Cazillis, M. (1973) J. Biol. Chem. 248, 131-137.
23. Chauveau, J., Moulé, Y. , and Rouiller, C. (1956) Exp. Cell Res. 11, 317-321.
24. Aaronson, R.P. , and Blobel, G. (1974) J. Cell Biol. 62, 746-754.
25. Martin, R.G. and Ames, B.N. (1961) J. Biol. Chem. 236, 1372-1379.
26. Holmes, A.M., Hesslewood, I.P. and Johnston, I.R. (1974) Eur. J. Biochem. 43, 487-499.
27. Hecht, N.B. (1975) Biochim. Biophys. Acta, 383, 388-398.
28. Morioka, K., and Terayama, H. (1974) Biochem. Biophys. Res. Commun. 61, 568-575.
29. Stalker, D.M., Probst, G.S., Mosbaugh, D.M., Kunkel, T.A. and Meyer, R.R. (1974) J. Cell Biol. 63, 332a.