The Occurrence of Two Types of Deoxyribonucleic Acid Polymerase Activity in the Main Classes of Nuclei obtained from the Brains of Infant and Adult Rats

By M. A. STAMBOLOVA, D. COX and A. P. MATHIAS
Wellcome Laboratories, Department of Biochemistry, University College London,
Gower Street, London WC1E 6BT, U.K.

(Received 25 October 1973)

1. The influence of exogenous or activated DNA template on the DNA polymerase activity in the different types of intact nuclei from rat brain tissue was determined. The different amounts or physical state of the DNA template did not produce significant differences in the relative distribution of the DNA polymerase activity between the separate groups of nuclei. 2. The DNA polymerase activities, fractionated by sucrose gradient centrifugation into enzyme A and enzyme B, were found to be present in the extracts of all types of rat brain nuclei. The distribution of these two activities in the 'particulate' and 'soluble' fractions of the separate groups of nuclei from 10-day-old and adult rats was studied. The findings are related to the DNA-synthetic activity *in vivo* of the intact nuclei and the possible biological functions of the DNA polymerase activities are discussed.

The DNA polymerase activity in nuclei from rat brain has been subjected to numerous investigations (Chiu & Sung, 1972*a,b*, 1973; Murthy & Bharucha, 1971; Bharucha & Murthy, 1971; Shimada & Terayama, 1972; Mathias & Stambolova, 1973; Stambolova *et al.*, 1973). The enzyme has been characterized (Chiu & Sung, 1971; Shimada & Terayama, 1972; Stambolova *et al.*, 1973) and partially purified (Chiu & Sung, 1972*a,b*). The distribution of its activity in the different regions of rat brain (Chiu & Sung, 1972*b*) and in the different types of brain nuclei (Stambolova *et al.*, 1973) has also been studied. In our previous papers, we have reported that the DNA polymerase activity of the

different types of nuclei, measured *in vitro*, was not related to the rate of DNA synthesis *in vivo* in so far as the latter can be measured by determining the extent of incorporation of radioactive thymidine (injected intracisternally or intraperitoneally) into nuclear DNA. We have suggested that this discrepancy could be ascribed to the different physical states of the DNA in the separate fractions of nuclei or to the excess of exogenous template used in the assay systems *in vitro*. Alternatively, the enzyme activity measured *in vitro* could represent the total replication and repair activity and would not therefore give a true indication of the replicative system unless subjected to further separation. Further, the

Table 1. Effect of the DNA template on the DNA polymerase activity in 10-day-old rat brain nuclei fractionated by zonal centrifugation

Nuclei purified by centrifugation through 2.0M-sucrose, 1 mM with respect to MgCl₂, 1 mM-sodium cacodylate buffer, pH 6.4, at 75000*g* for 60 min at 5°C were fractionated on a discontinuous gradient ranging from 2.0M to 2.8M-sucrose. Zone (I) contains the nuclei banding at the interface between 2.0 and 2.3M-sucrose, zone (II) 2.3-2.4M-sucrose, zone (III) 2.4-2.5M-sucrose, zone (IV) 2.5-2.6M-sucrose and zone (V) 2.6-2.8M-sucrose. The nuclei from each zone were recovered by dilution of the peak fractions with 0.32M-sucrose, 1 mM with respect to MgCl₂, pH 6.4, followed by centrifugation.

Zones	Template ...	DNA polymerase activity (units/mg of DNA)		
		Native DNA*	Activated DNA	DNA omitted
I		26.7	33.0	3.6
II		28.0	27.6	2.7
III		15.0	13.8	1.6
IV		11.9	9.7	1.3
V		7.9	9.3	1.3

* Stambolova *et al.* (1973).

Fig. 1. Sucrose-density-gradient centrifugation of DNA polymerase from fractionated brain nuclei of 10-day-old rats

Rat brain nuclei from 80g of brain tissue were fractionated by zonal centrifugation and sequentially extracted with 0.01 M-Tris-HCl buffer, pH 7.4, 2 mM- β -mercaptoethanol ('soluble' DNA polymerase) and 0.2 M-sodium phosphate buffer (pH 7.4)-2 mM- β -mercaptoethanol ('particulate' DNA polymerase). The extracts were concentrated by using Hollow Fibre Ultrafilters and sucrose-density-gradient sedimentation patterns were obtained as described in the Materials and Methods section. —, Tris buffer extract; ----, phosphate buffer extract; (○), native DNA template; (Δ), denatured DNA template. (a) Neuronal nuclei (zone I); (b) astrocytic nuclei (zone II-III); (c) oligodendrocyte nuclei (zone IV and V). The direction of sedimentation is from right to left.

experiments *in vivo* may be complicated by differences in the uptake of thymidine and the size of the dTTP pool between the many types of brain cells.

To clarify this situation we have investigated the effect of adding either native or denatured DNA to the incubation mixture used for the assay *in vitro* of DNA polymerase. In addition we have exploited the observation of Chiu & Sung (1972a,b), who found that the DNA polymerase of unfractionated rat brain nuclei could be separated by density-gradient centrifugation into two types. Enzyme 'A' has a sedimentation coefficient of 9S, a preference for denatured DNA and may be required for replication of DNA; enzyme 'B' sediments around 4S, prefers native DNA and has an unknown role but possibly is required for repair synthesis. We have studied the

distribution of A and B enzymes in the different sorts of brain nuclei and its relationship to the nuclei active in DNA synthesis *in vivo*.

Materials and Methods

Reagents

Unlabelled deoxyribonucleoside triphosphates were obtained from Boehringer Corp (London) Ltd., Ealing, London W5 2TZ, U.K. [3 H]Thymidine 5'-triphosphate (sp. radioactivity 23.9 Ci/mmol) was supplied by The Radiochemical Centre, Amersham, Bucks., U.K. Calf thymus DNA (type 1) was purchased from Sigma (London) Chemical Co., London S.W.6, U.K.

Fig. 2. Sucrose-density-gradient centrifugation of DNA polymerase from fractionated brain nuclei of adult rats

Nuclei (from 80 g of brain tissue) were fractionated, extracted and sucrose-density-gradient sedimentation patterns were obtained as described in Fig. 1. The nuclear extracts were dialysed against 0.01 M-Tris-HCl buffer, pH 7.4, containing 2 mM- β -mercaptoethanol and concentrated by the use of UM-10 Amicon Ultrafilters before the sucrose gradient fractionation. —, Tris buffer extract; ----, phosphate buffer extract. (a) Neuronal nuclei (zone I); (b) astrocytic nuclei (zone II-III); (c) oligodendrocyte nuclei (zone IV and V).

Animals

Infant (20 g) and young adult (90 g) Wistar rats were used.

Isolation and fractionation of the nuclei

The animals were killed and the nuclei were isolated and fractionated by zonal centrifugation as previously described (Stambolova *et al.*, 1973).

Extraction and fractionation of the 'soluble' and 'particulate' forms of DNA polymerases

The pooled fractions containing the different types of nuclei were extracted within 1 week, during which time they were kept in 2.0 M-sucrose, 1 mM with respect to $MgCl_2$, pH 6.4, at $-20^\circ C$. Control experiments showed that storage of the nuclei under these conditions caused negligible loss of either form of DNA polymerase. The DNA polymerases were

extracted and fractionated by the method of Chiu & Sung (1972a). Brain nuclei were separated from brain tissue (approx. 80 g), extracted and the DNA polymerases examined by density-gradient centrifugation. The extraction procedure is outlined in the legend to Fig. 1.

The 'soluble' and 'particulate' fractions of the DNA polymerases from each type of nucleus were concentrated where necessary by use of Amicon or Hollow Fibre Ultrafilters. Hollow Fibre Ultrafilters were supplied by Bio-Rad Laboratories Ltd., St. Albans, Herts., U.K. The concentration was achieved with 'Bio Fibre 80 T-tube' filters at a flow rate of 0.8 ml of enzyme extract/min.

Samples (0.4 ml; approx. 1.6 mg of protein) were layered over 5–20% (w/v) sucrose gradients in 5 ml cellulose nitrate tubes and centrifuged for 14 h in a SW 50.1 rotor in a Spinco model L265B ultracentrifuge at 36000 rev./min, at $0^\circ C$. After centrifugation the tubes were punctured, seven-drop

($\approx 110 \mu\text{l}$) fractions collected and each fraction was assayed for DNA polymerase activity with native or denatured DNA as template (in a total volume of 0.25 ml) as previously described (Stambolova *et al.*, 1973).

One unit of DNA polymerase activity was defined as the incorporation of 1 pmol of [^3H]dTTP into acid-insoluble product in 1 min, at 37°C. Specific activity was expressed as units per mg of DNA. Denatured DNA was prepared by heating the DNA solution for 10 min at 100°C in 0.01 M-NaCl and immediately cooling in ice. Activation of DNA was carried out by the method of Aposhian & Kornberg (1962). DNA was measured by the diphenylamine reaction as modified by Croft & Lubran (1965). Protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Results and Discussion

We have shown that the total DNA polymerase activity measured *in vitro* is highest in the nuclei from zone (I) (predominantly neuronal nuclei) and exhibits a progressive decline in the following zones, whereas the DNA synthesis *in vivo* was of the same order in the first three fractions and increased considerably in zones (IV) and (V) (mainly oligodendrocyte nuclei) (Mathias & Stambolova, 1973; Stambolova *et al.*, 1973). DNA polymerase activity of the different types of nuclei was investigated in the absence of exogenous DNA and after the addition of activated template to the assay system (Table 1). It was found that the omission of exogenous DNA had a restrictive effect on the enzyme activity of the different nuclei. The incorporation of [^3H]dTTP was about 10% of the uptake in the complete system. However, the absence of added DNA did not produce any significant difference in the relative distribution of the DNA polymerase activity between the separate groups of nuclei. The same pattern of relationship of the enzyme activities was observed when activated DNA was used (Table 1). The inclusion of exogenous DNA in the assay for DNA polymerase obviously does not distort the relative activities of the nuclei in the five separated zones and cannot be an explanation of the discrepancy between results *in vivo* and *in vitro*.

The studies of Chiu & Sung (1972b, 1973) have shown that, in common with other mammalian systems (Chang & Bollum, 1971), there are at least two forms of DNA polymerase in rat brain nuclei. In 10-day-old rats 60% of the DNA polymerase activity is cytoplasmic and mainly in the 'A' form, whereas the remaining 40% is in the nucleus and is predominantly in 'B' form. In adult rats only 20% of the activity is found in the cytoplasm, equally divided between forms 'A' and 'B', the bulk of the activity being in the nuclei mainly as form 'B'. In view of the

different relative activities of total DNA polymerase in the main types of brain nuclei, we have examined the amounts of these two nuclear polymerases in each of the five peaks from the zonal fractionation of the brain nuclei (Figs. 1 and 2). The two forms of DNA polymerase, first described by Chiu & Sung (1972b) in brain nuclei, are present in all types of nuclei from both 10-day-old and adult rats. The A and B forms of the enzyme were found in the so-called 'soluble' or Tris-HCl extract and also in the 'particulate' or phosphate buffer extract. The larger part of the total DNA polymerase activity of all nuclear fractions, with the exception of the zones (IV) and (V) (oligodendrocyte) nuclei from infant rats (Fig. 1c) was due to the action of enzyme B, which was mainly in 'particulate' form. The changes in enzyme activity between the different types of nuclei corresponded in general to those which we had observed in the total DNA polymerase activity of the separate zones of intact nuclei (Stambolova *et al.*, 1973).

However, it is significant that the soluble activity of enzyme A is greatest in the zone (IV) and zone (V) nuclei and least in the zone (I) nuclei (Fig. 1). This parallels the ability of the nuclei to incorporate [^3H]thymidine *in vivo* (Stambolova *et al.*, 1973). In 10-day-old rats zone (IV) and (V) nuclei incorporate *in vivo* two to three times the radioactivity found in zone (I) nuclei. Thus the activity of the A enzyme in the Tris-HCl extract in the different types of nuclei reflects reasonably closely the ability to synthesize DNA *in vivo*. The high activity in zone (V) may be due to the presence in this fraction of microneuronal nuclei, most of which may be of postnatal origin (Altman, 1966). In adult rats the activity of form B of the extract prepared with phosphate buffer remains comparatively high, whereas the activity in the Tris-HCl extract is markedly diminished.

These findings in the fractionated nuclei support the view (Chiu & Sung, 1972b) that the DNA polymerase activity in the rat brain nuclei, in common with other mammalian systems (Chang & Bollum, 1971), is attributable to two enzymes: enzyme 'A', possibly responsible for the DNA replication, and enzyme 'B', with an unknown biological role. The experimental results are consistent with our previous suggestion (Stambolova *et al.*, 1973) that the total DNA polymerase activity measured *in vitro* represents replication and additional activities (e.g. the repair activity of the nuclei). Hence the assay cannot give a true indication of the system for replication.

The increased activity of DNA polymerase 'A' in the nuclei active in DNA synthesis can be considered as evidence for the involvement of this enzyme in the process of replication and this argument is supported by the parallel incorporation of thymidine and the enzyme activity. However, conclusions concerning the complex enzymology of the replication of DNA also require an exhaustive study of the cell

proteins whose co-operation is undoubtedly necessary for the full expression of the enzyme activity.

This work was supported by the Science Research Council and carried out during the tenure of a Fellowship from the Food and Agricultural Organisation granted to M. A. S. We thank Mr. David Ridge for advice about the zonal centrifugation.

References

- Altman, J. (1966) *J. Comp. Neurol.* **128**, 431–474
- Aposhian, H. V. & Kornberg, A. (1962) *J. Biol. Chem.* **237**, 519–525
- Bharucha, A. D. & Murthy, M. R. V. (1971) *Can. J. Biochem.* **49**, 978–986
- Chang, L. M. S. & Bollum, F. J. (1971) *J. Biol. Chem.* **246**, 5835–5837
- Chiu, J. F. & Sung, S. C. (1971) *Biochim. Biophys. Acta* **246**, 44–50
- Chiu, J. F. & Sung, S. C. (1972a) *Biochim. Biophys. Acta* **262**, 397–400
- Chiu, J. F. & Sung, S. C. (1972b) *Biochim. Biophys. Acta* **269**, 364–369
- Chiu, J. F. & Sung, S. C. (1973) *J. Neurochem.* **20**, 617–619
- Croft, D. N. & Lubran, M. (1965) *Biochem. J.* **95**, 612–620
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mathias, A. P. & Stambolova, M. A. (1973) *Biochem. Soc. Trans.* **1**, 624–626
- Murthy, M. R. V. & Bharucha, A. D. (1971) *Can. J. Biochem.* **49**, 1285–1291
- Shimada, H. & Terayama, H. (1972) *Biochim. Biophys. Acta* **287**, 415–426
- Stambolova, M. A., Cox, D. & Mathias, A. P. (1973) *Biochem. J.* **136**, 685–695