Deoxyribonucleic Acid Polymerases of BHK-21/C13 Cells

HETEROGENEITY, MOLECULAR ASYMMETRY AND SUBCELLULAR DISTRIBUTION OF THE ENZYMES

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Nuclear and cytoplasmic fractions were prepared from exponentially-growing BHK-21/C13 cells; DNA polymerase was extracted from them and analysed by gel filtration and sucrose-density-gradient centrifugation. DNA polymerase I is heterogeneous comprising species covering a considerable range of molecular weights. These have been tentatively identified as four subspecies of apparent molecular weights 900 000–1 000 000 (IA), 460 000–560 000 (IB), 270 000–320 000 (IC) and 140 000–200 000 (ID), as assessed by gel filtration through Sepharose 6B. DNA polymerase II has a mol.wt. of $46\,000 \pm 4\,000$ as assessed by gel filtration on Sepharose 6B, and $48\,000 \pm 2\,000$ as assessed by gel filtration on Sephadex G-100. Sedimentation analyses on sucrose density gradients showed that the DNA polymerase I species had sedimentation coefficients predominantly in the range 6–8S. DNA polymerase II had predominantly a sedimentation coefficient of 3.2S although a component with lower sedimentation coefficient was found. The lack of correlation between the molecular weights derived from gel filtration and the sedimentation coefficients is attributed to molecular asymmetry. DNA polymerase I was found to be associated predominantly with the cytoplasm although certain types of nuclear preparation contained large amounts of it. DNA polymerase II was found to be mostly if not exclusively in nuclear preparations.

Two species of replicative DNA-dependent DNA nucleotidyltransferases (EC 2.7.7.7; hereafter termed DNA polymerases) have been partially purified and characterized from extracts of exponentially growing BHK-21/C13 cells (Craig & Keir, 1975); similar species have been identified from a number of tissues and cell lines (for review see Craig & Keir, 1974). A high-molecular-weight enzyme (sedimentation coefficient 6–8S; DNA polymerase I), itself displaying considerable heterogeneity, has been isolated, primarily from the cytoplasm of a variety of proliferating cell lines and tissues (Craig & Keir, 1974). A low-molecular-weight enzyme (sedimentation coefficient 3.3S; DNA polymerase II) has been identified both in actively replicating and in differentiating or quiescent tissues and cell lines, and appears to be primarily of nuclear origin (Craig & Keir, 1974).

The present paper describes the apparent subcellular distribution of DNA polymerases I and II in exponentially growing BHK-21/C13 cells, and also demonstrates the heterogeneous nature of DNA polymerase I (the 6–8S species).

Experimental

Materials

Buffers were prepared as described by Craig & Keir (1975). Buffer A was 10mM-Tris-HCl (pH 7.5 at 4°C)–5mM-2-mercaptoethanol. Buffer B was 0.2M-potassium phosphate (pH 7.5)–5mM-2-mercaptoethanol. Buffer C was 0.1M-potassium phosphate (pH 7.5)–5mM-2-mercaptoethanol. TD buffer was 25mM-Tris-HCl (pH 7.5 at 4°C)–0.14M-NaCl–0.05M-KCl–0.2M-Na₂HPO₄.

Sephacrose 6B was purchased from Pharmacia (G.B.) Ltd., London W5 5SS, U.K.; urease was from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey KT2 7BH, U.K.; and catalase was from Boehringer Corp. (London) Ltd., London W5 2TZ, U.K. Other materials were as described by Craig & Keir (1975).

Methods

Cell culture and preparation of 'cytoplasmic' and 'nuclear' fractions. Cells in the exponential phase of growth were harvested by using either 0.5mM-EDTA in phosphate-buffered saline, or trypsin-Tris-saline,

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as described by Craig & Keir (1975) and Craig *et al.* (1975). All operations thereafter were at 0–4°C. The cells were then washed by resuspension in TD buffer (10ml/bottle of cells) followed by sedimentation at 200g_{av.} for 5 min. The cells were then finally resuspended in hypo-osmotic buffer A (5ml/bottle of cells) and allowed to swell for 5 min. They were disrupted, as described by Craig & Keir (1975), in a glass homogenizer tube with a Teflon pestle. After centrifugation at 800g_{av.} for 10 min, the resulting supernatant fraction (the 'low-speed cytoplasm') was retained, and the sediment, consisting predominantly of nuclei and some cell debris, was resuspended in buffer A containing 0.32M-sucrose (5ml/bottle of cells).

Purification of nuclei. (1) Sedimentation through sucrose solutions. Exponentially growing BHK-21/C13 cells (six bottles) were harvested by using 0.5mM-EDTA in phosphate-buffered saline, washed and homogenized as described above. The resulting sediment was resuspended in 30ml of buffer A containing 0.32M-sucrose, divided into three 10ml portions each of which was layered on 10ml of buffer A containing 1.2M-sucrose in a 23ml polypropylene centrifuge tube. The nuclei were then sedimented by centrifugation at 12000g_{av.} for 20 min in the 3×23ml aluminium swing-out rotor on an MSE Superspeed 65 ultracentrifuge. The debris accumulated at the interface of the two sucrose layers was aspirated off, and the nuclei were resuspended in 30ml of buffer A containing 1.2M-sucrose. The nuclear suspension was then layered as described above on buffer A containing 2.0M-sucrose, and the nuclei were again sedimented by centrifugation in the 3×23ml rotor at 75000g_{av.} for 60 min. Cellular debris and sucrose were then removed, and the resulting translucent nuclear pellets used as a source of nuclear DNA polymerase activity. Examination by electron microscopy demonstrated the virtual absence of contaminating subcellular components. Contamination was limited to what appeared to be ribosomes attached to the outer nuclear membrane. The nuclei themselves were somewhat swollen and ruptured, but otherwise clean and discrete.

(2) Repeated washing in buffer solution. The initial sediment (nuclei and cell debris) obtained after homogenization was resuspended in buffer A containing 0.32M-sucrose (5ml/bottle of cells) and was resedimented by centrifugation at 800g_{av.} for 10 min. The supernatant was discarded and the sediment washed twice in buffer A containing 0.32M-sucrose as described above. The resulting nuclei on examination by phase-contrast light microscopy appeared intact and essentially free from cytoplasmic tags and general cellular debris. These nuclei were then used as a source of 'nuclear' DNA polymerase activity.

Preparation of cytoplasmic and nuclear extracts for ion-exchange chromatography and gel-filtration. Purified nuclei and 'low-speed cytoplasmic' fractions were treated in a manner identical with that described by Craig & Keir (1975). In brief, the purified nuclei were suspended in buffer B (2.5ml/bottle of cells) and left for 1 h. High-speed centrifugation of both the 'low-speed cytoplasm' and the 'nuclear' extracts yielded supernatant fractions which were concentrated by precipitation with (NH₄)₂SO₄. When the resulting concentrated fractions were to be used as a source of enzyme for fractionation by gel filtration, the precipitates were dissolved in a minimal volume of buffer B. When ion-exchange chromatography on columns of phosphocellulose preceded gel filtration, then the precipitates were dissolved in buffer C. This preparative methodology ensured removal of insoluble cell debris, microsomal fraction, ribosomes and mitochondria from the cytoplasmic fraction, before exposure of it to the hyperosmotic buffer B and buffer C, and to high concentration of (NH₄)₂SO₄.

Column chromatography. (1) Phosphocellulose. Cytoplasmic or nuclear extracts (the latter from nuclei purified by extensive washing with buffer A containing 0.32M-sucrose) obtained from approx. 4×10⁹ cells (20 bottles) were loaded at 10ml/h on to a column (1.7cm×9cm) of phosphocellulose equilibrated with buffer C. Unbound protein was removed by extensive washing, and a stepwise elution was carried out with 0.4M-potassium phosphate buffer, pH 7.5, containing 5mM-2-mercaptoethanol (Craig & Keir, 1975). Fractions (2ml) were collected, and those containing DNA polymerase activity were pooled and dialysed against buffer C at 4°C.

(2) Sephadex G-100. The appropriate cytoplasmic or nuclear dialysed preparation (3ml) from chromatography on phosphocellulose was applied to a calibrated (Craig & Keir, 1975) column (1.2cm×86cm; 130ml bed volume) of Sephadex G-100 equilibrated with buffer A. Fractions (2.0ml) were collected at a flow rate of 5ml/h and assayed for DNA polymerase activity at both pH 7.6 and 8.9. The column was calibrated for estimations of molecular weight (Andrews, 1965) by using Blue Dextran, lactate dehydrogenase, alcohol dehydrogenase, ovalbumin and cytochrome c as standards.

(3) Sepharose 6B. Gel filtration of a dialysed cytoplasmic preparation from chromatography on phosphocellulose (3ml) was applied to a calibrated column (1.2cm×86cm; 130ml bed volume) of Sepharose 6B equilibrated in buffer C. Fractions (2.8ml) were collected at a flow rate of 10ml/h and assayed for DNA polymerase activity at both pH 7.6 and 8.9.

When column chromatography on phosphocellulose was omitted, fractions (2.0ml) of the appropriate dialysed cytoplasmic or nuclear (nuclei purified

by centrifugation through sucrose) fractions, were applied to the same calibrated column of Sepharose 6B, equilibrated in buffer B. Fractions (2.8 ml) were collected at a flow rate of 10 ml/h and assayed for DNA polymerase activity at pH 7.6. The column was calibrated for molecular-weight estimations (Andrews, 1965) by using Blue Dextran and the following standard proteins: urease [jack bean; mol.wt. 483 000 (Creeth & Nichol, 1960)], catalase [bovine liver; mol.wt. 250 000 (Samejima & Yang, 1963)], lactate dehydrogenase [pig heart; mol.wt. 140 000 (Goldberg, 1972)], ovalbumin [hen; mol.wt. 45 000 (Cunningham *et al.*, 1963)] and cytochrome *c* [horse heart; mol.wt. 12 400 (Margoliash, 1962)].

The marker enzymes were assayed by standard techniques: urease by the method of Sumner (1955), catalase by the method of Chance & Maehly (1955) and lactate dehydrogenase by the method of Kornberg (1955). Blue Dextran, ovalbumin and cytochrome *c* were monitored in column eluates spectrophotometrically at 650, 280 and 412 nm respectively.

Sucrose-density-gradient centrifugation. Sucrose density gradients [4.8 ml; 5–20% (w/v)] were prepared in buffer B or C, by the freeze-thaw method of Baxter-Gabbard (1972). The polymerase preparation (200 μ l) and an enzyme marker [lactate dehydrogenase (20 μ l)] were applied to the top of the gradients, and centrifugation was carried out (13 or 16 h; 130 600 g_{av} , 4°C) in a Beckman-Spinco L2 ultracentrifuge in the SW50L rotor. Fractions (nine drops each) were collected by upward displacement of the gradient with 40% (w/v) sucrose, and assayed for DNA polymerase (under optimal conditions for DNA polymerase I) and lactate dehydrogenase activities. Sedimentation coefficients were determined by the method of Martin & Ames (1961).

Assay for DNA polymerase. Assays for DNA polymerases I and II were carried out as described by Craig & Keir (1975) with 'activated' DNA (Loeb, 1969) from salmon sperm as primer-template. Polymerase activity was expressed as nmol of [3 H]dTMP residues incorporated/30 min per fraction at 37°C. To ensure constant phosphate concentration in the assays, samples (20 μ l) of fractions in buffer B were assayed in a final volume of 200 μ l, and samples (20 μ l) of fractions in buffer A were assayed in a final volume of 100 μ l.

Results

DNA polymerase species associated with the cytoplasmic fraction

The heterogeneous nature of BHK cell DNA polymerase I has already been demonstrated by sucrose-density-gradient centrifugation (Craig & Keir, 1975). In an extension of these investigations

Fig. 1. Gel filtration of DNA polymerases from cytoplasm

The cytoplasmic preparation was first purified by chromatography on phosphocellulose. The fractions containing DNA polymerase activity were submitted to gel filtration through Sepharose 6B equilibrated with buffer C (see the Experimental section). ○, DNA polymerase activity at pH 7.6; ●, DNA polymerase activity at pH 8.9; V, void volume; LDH, lactate dehydrogenase; Ov, ovalbumin; cyt *c*, cytochrome *c*.

a cytoplasmic extract (from cells harvested in 0.5 mM-EDTA in phosphate-buffered saline) was submitted to gel filtration on Sepharose 6B after column chromatography on phosphocellulose. The elution profile (Fig. 1) not only confirmed the heterogeneous nature of DNA polymerase I [covering a wide range of molecular weights (see Fig. 3)] but also revealed the presence of DNA polymerase II in the cytoplasmic extract as judged by the criteria of pH and an elution volume corresponding to a molecular weight of $46\,000 \pm 4\,000$. DNA polymerase I was resolved into two major species, IA and IB, with molecular weights of 900×10^3 – 1000×10^3 and 460×10^3 – 560×10^3 respectively. The skewed profile of these major DNA polymerase I components suggested the possible presence of other species of lower molecular weight. Analysis of the same extract on a column of Sephadex G-100, produced a profile similar to that obtained for a total cell extract (Craig & Keir, 1975). The major activity, DNA polymerase I, displayed optimal activity at pH 7.6, being eluted as a skewed peak with the void volume, but a small but distinct peak of activity, showing optimal activity at pH 8.9, was eluted at exactly the same position as DNA polymerase II in the total cell preparation (mol.wt. $48\,000 \pm 2\,000$).

Further analysis of the same extract on a calibrated column of Sephadex G-200 (R. K. Craig & H. M. Keir, unpublished observations) also demonstrated the heterogeneity of DNA polymerase I. Although the major portion of the activity was eluted with the void volume (DNA polymerase IA), a distinct shoulder of activity (DNA polymerase IB) was

Fig. 2. Gel filtration of DNA polymerase from cytoplasm

Cells were harvested in trypsin-Tris-saline solution, and the cytoplasmic extract was applied directly to a column of Sepharose 6B equilibrated with buffer B, without prior chromatography on phosphocellulose. Fractions were assayed under conditions optimal for DNA polymerase I.

Fig. 3. Molecular weights of DNA polymerases from BHK-21/C13 cells

The elution volume from the column of Sepharose 6B is plotted against the logarithm of the molecular weight of the following standards; urease, catalase, lactate dehydrogenase (LDH), ovalbumin (ov) and cytochrome *c* (cyt *c*). Blue Dextran emerged in the void volume.

eluted just after the void volume, corresponding to an estimated molecular weight of 500×10^3 – 550×10^3 .

When the cells were harvested in trypsin-Tris-saline solution, and the cytoplasmic extract was submitted to gel filtration on Sepharose 6B in buffer B, with the omission of the phosphocellulose column chromatography step, the heterogeneous nature of DNA polymerase I was again revealed (Fig. 2), there being a major peak corresponding to DNA poly-

Fig. 4. Sedimentation analysis of cytoplasmic DNA polymerase by sucrose-density-gradient centrifugation

A portion of the cytoplasmic enzyme preparation that had been analysed by gel filtration through Sepharose 6B (Fig. 2) was submitted to centrifugation (16h) on a sucrose density gradient prepared in buffer B (see the Experimental section). Fractions were assayed for lactate dehydrogenase (LDH) and DNA polymerase I.

merase IA, a distinct shoulder corresponding to DNA polymerase IB, and minor shoulders corresponding to DNA polymerases IC and ID. The latter corresponded to apparent molecular weights of 270×10^3 – 320×10^3 and 140×10^3 – 200×10^3 respectively (Fig. 3). No activity corresponding to DNA polymerase II could be detected. When a portion of the same extract was analysed by sucrose-density-gradient centrifugation in buffer B, the activity profile (Fig. 4) again revealed heterogeneity, the major polymerase activity sedimenting with a sedimentation coefficient of 7.2S; there were also distinct shoulders of activity corresponding to sedimentation coefficients of 4.4S and 5.9S. No activity corresponding to a sedimentation coefficient in the region of 3.3S was detected.

The molecular weights of the cytoplasmic DNA polymerases I (Figs. 1 and 2) as estimated by gel filtration through Sepharose 6B, clearly do not correlate with molecular weights derived from sedimentation coefficients (Fig. 4). This is presumably attributable to asymmetry of the DNA polymerase subunit complex (see the Discussion section).

Fig. 5. Sedimentation analyses of cytoplasmic DNA polymerase fractions after gel filtration

DNA polymerase fractions IA, IB and IC obtained from the gel filtration on Sepharose 6B (Fig. 2) were concentrated 10-fold by pressure dialysis and centrifuged on sucrose density gradients prepared in buffer B (see the Experimental section) for 13 h. Fractions were assayed for DNA polymerase I (○) and lactate dehydrogenase (LDH; ●).

In an attempt to correlate directly molecular weights as estimated by gel filtration through Sepharose 6B with sedimentation coefficients, the peak fractions corresponding to polymerases IA, IB and IC from gel filtration (Fig. 2) were submitted to sedimentation analysis on sucrose gradients. They were concentrated by pressure dialysis in a Sartorius collodion bag mounted in a suction vessel containing buffer B. Samples of each concentrate were then analysed by centrifugation for 13 h on 5–20% sucrose density gradients in buffer B. The activity profiles (Fig. 5) bore little resemblance to the total

Fig. 6. Gel filtration of DNA polymerases from nuclei purified by centrifugation through sucrose solution

The nuclei were purified by centrifugation through 2.0M-sucrose, and the extract from them was concentrated by precipitation with $(\text{NH}_4)_2\text{SO}_4$ before fractionation on the column of Sepharose 6B equilibrated with buffer B. Assays for DNA polymerase were performed under conditions optimal for polymerase I.

cytoplasmic enzyme profile (Fig. 4). After concentration, DNA polymerase IA revealed a single major peak of activity (sedimentation coefficient 7.6S), with an additional shoulder of activity at 5.8S (Fig. 5a). DNA polymerase IB (an enzyme of apparently half the molecular weight of DNA polymerase IA as analysed by gel filtration) revealed a bimodal distribution, with a major peak at 8.5S and a substantial shoulder of activity corresponding to 7.3S (Fig. 5b). DNA polymerase IC similarly revealed a bimodal distribution, the major activity running at 7.6S with a shoulder of activity at 8.3S (Fig. 5c). No activities corresponding to sedimentation coefficients of less than 5.8S were observed.

DNA polymerase species associated with the nuclear fraction

BHK-21/C13 cells were harvested in 0.5mM-EDTA in phosphate-buffered saline, and the nuclei subsequently purified through 2.0M-sucrose, extracted with buffer B; the extract was concentrated by $(\text{NH}_4)_2\text{SO}_4$ precipitation and fractionated by gel filtration in Sepharose 6B (Fig. 6). The profile clearly revealed that DNA polymerase II (mol.wt. 46000 ± 4000) was the predominant species and represented 80% of the total polymerase recovered. The remaining activity consisted of DNA polymerase IA (mol.wt. 900000–1 100000) and DNA polymerase IC (mol.wt. 270000–320000).

However, if the nuclei again from cells harvested in 0.5mM-EDTA in phosphate-buffered saline were

Fig. 7. Gel filtration of DNA polymerases from nuclei purified by repeated washing

The extract from the washed nuclei was concentrated by precipitation with $(\text{NH}_4)_2\text{SO}_4$, fractionated on phosphocellulose then analysed by gel filtration through Sephadex G-100 equilibrated with buffer C. $\circ$, DNA polymerase I activity at pH 7.6; $\bullet$, DNA polymerase activity at pH 8.9.

simply purified by washing in buffer A containing 0.32M-sucrose, followed by extraction with buffer B, concentration by $(\text{NH}_4)_2\text{SO}_4$, phosphocellulose chromatography and finally gel filtration through Sephadex G-100 (Fig. 7), then DNA polymerase II contributed only 27% of the total activity recovered (assayed at pH 7.6); the remainder consisted of the DNA polymerase I species which were eluted with the void volume.

In order to correlate the molecular weight of DNA polymerase II, as obtained by gel filtration of the purified nuclear extract (Fig. 6) with the same molecular-weight species from a variety of tissues and cell lines, as obtained by sedimentation analysis on glycerol or sucrose density gradients (see Craig & Keir, 1974), a sample of nuclear extract was analysed by sucrose-density-gradient centrifugation. The result (Fig. 8) was unexpected. Although no DNA polymerase activity ran in the region of the lactate dehydrogenase marker (7.3S; DNA polymerase I, 6–8S), and although most of the activity recovered sedimented as expected with a sedimentation coefficient of 3.2S, a sharp front of DNA polymerase activity was observed, corresponding to a sedimentation coefficient of 1.8S.

Discussion

Investigation into the subcellular distribution of DNA polymerases in BHK-21/C13 cells, not only confirms our previous findings that DNA polymerase

Fig. 8. Sedimentation analysis of nuclear DNA polymerase by sucrose-density-gradient centrifugation

A portion of the nuclear enzyme preparation that had been analysed by gel filtration through Sepharose 6B (Fig. 1) was submitted to sedimentation analysis on sucrose gradients prepared in buffer B (see the Experimental section). Fractions were assayed for DNA polymerase I and lactate dehydrogenase (LDH).

I shows considerable heterogeneity (Craig & Keir, 1975), but that DNA polymerase I is clearly closely associated with the cell nucleus. Whether BHK cell DNA polymerase I is associated with the nuclear membrane, and is subsequently inactivated during purification procedures involving high concentrations of sucrose, salt or detergent (see Lazarus & Kitron, 1973), remains to be proven. However, it is evident that whereas DNA polymerase I can be identified from extracts of nuclei purified through sucrose, followed by analysis by gel filtration through Sepharose 6B, no activity corresponding to DNA polymerase I (6–8S) could be detected when the same nuclear extract was analysed by sucrose-density-gradient centrifugation (Fig. 8), in spite of the fact that all assays were performed under conditions optimal for DNA polymerase I. Such analysis did reveal polymerase activity with a sedimentation coefficient of 1.8S in addition to the major activity sedimenting at 3.2S. It is not known whether this is a distinct DNA polymerase species. A similar activity has been observed in total cell extracts obtained from quiescent cells [cells maintained in medium containing only 0.5% (v/v) instead of 10% (v/v) calf serum (R. K. Craig & H. M. Keir, unpublished work)] after sucrose-

density-gradient analysis. Its apparent absence from extracts analysed by gel filtration could presumably be due to the poor resolving power of Sepharose 6B for molecules lying within the lower range of molecular weights. No such activity could be detected in fractions which had undergone chromatography on phosphocellulose. The detection of DNA polymerase activities with sedimentation coefficients of a similar low value has been reported from embryonic muscle (Wicha & Stockdale, 1972) and chick embryo (Brun *et al.*, 1974).

DNA polymerase I is the major if not the only cytoplasmic species. In the light of recent work based on the interconvertibility of the 6-8S and 3.3S enzymes from mouse testis (Hecht, 1973; Hecht & Davidson, 1973) and rat liver (Tanabe & Takahashi, 1973), it seems possible that the apparent presence of DNA polymerase II in the cytoplasm might be the consequence of partial dissociation of the high-molecular-weight DNA polymerase I into the low-molecular-weight DNA polymerase II during elution with 0.4M-potassium phosphate buffer from the phosphocellulose column. Lazarus & Kitron (1973) also working with BHK cell DNA polymerases, reported the partial conversion at high ionic strength of high-molecular-weight DNA polymerase I of the cytoplasm into a low-molecular-weight enzyme resembling DNA polymerase II.

Although molecular-weight estimations of DNA polymerase II agree closely with those obtained for similar enzymes derived from rat liver (Haines *et al.*, 1972), KB cells (Sedwick *et al.*, 1972; Wang *et al.*, 1974) and calf thymus (Chang, 1973), those of DNA polymerase I components are different from published values from other systems (Craig & Keir, 1974). The detection of polymerase IA and IB (mol.wts. 900×10^3 – 1100×10^3 and 460×10^3 – 560×10^3 respectively) as major species agrees well with observations made on other cells or tissues (Harm & Hilz, 1971; Wallace *et al.*, 1971; Adams *et al.*, 1973; Holmes *et al.*, 1973), but our detection of the minor species IC and ID is the first demonstration of such marked heterogeneity in the polymerase I of enzyme within a single cell type.

This tentative identification of multiple species of DNA polymerase I is further substantiated by the identification of the same species during experiments designed to investigate the relationship of DNA polymerases I and II to the physiological state of the cell (Craig *et al.*, 1975). Although all of our data (Craig, 1974; Craig & Keir, 1975; Craig *et al.*, 1975) have clearly and consistently demonstrated the heterogeneous nature of DNA polymerase I by both gel filtration and sucrose-density-gradient centrifugation, there is an obvious discrepancy between the molecular weights estimated by gel filtration and those estimated by sedimentation analysis. Similar observations have

been reported for Ehrlich ascites-tumour cells (Harm & Hilz, 1971), rat liver (Holmes & Johnston, 1973) and calf thymus and rat spleen (Holmes *et al.*, 1973), the discrepancies being attributed to the non-standard behaviour of DNA polymerase I species during gel filtration, due to the apparent asymmetric nature of the enzyme or enzyme subunit complex. Our attempts to correlate DNA polymerase I subspecies with specific sedimentation coefficients proved inconclusive (Fig. 5), presumably because of aggregation (specific or otherwise) of the lower-molecular-weight subspecies during concentration of the fractions from Sepharose 6B before sedimentation analysis.

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References

- Adams, R. L. P., Henderson, M. A. L., Wood, W. & Lindsay, J. G. (1973) *Biochem. J.*, **131**, 237–246
- Andrews, P. (1965) *Biochem. J.* **96**, 595–606
- Baxter-Gabbard, K. L. (1972) *FEBS Lett.* **20**, 117–119
- Brun, G., Rougeon, F., Lauber, M. & Chapeville, F. (1974) *Eur. J. Biochem.* **41**, 241–251
- Chance, B. & Maehly, A. C. (1955) *Methods. Enzymol.* **2**, 764–775
- Chang, L. M. S. (1973) *J. Biol. Chem.* **248**, 3789–3795
- Craig, R. K. (1974) Ph.D. Thesis, University of Aberdeen
- Craig, R. K. & Keir, H. M. (1974) in *The Cell Nucleus* (Busch, H., ed.), vol. 3, pp. 35–66, Academic Press, London and New York
- Craig, R. K. & Keir, H. M. (1975) *Biochem. J.* **145**, 215–224
- Craig, R. K., Costello, P. A. & Keir, H. M. (1975) *Biochem. J.* **145**, 233–240
- Creeth, J. M. & Nichol, L. W. (1960) *Biochem. J.* **77**, 230–239
- Cunningham, L. W., Clouse, R. W. & Ford, J. D. (1963) *Biochim. Biophys. Acta* **78**, 379–381
- Goldberg, E. (1972) *J. Biol. Chem.* **247**, 2044–2048
- Haines, M. E., Wickremasinghe, R. G. & Johnston, I. R. (1972) *Eur. J. Biochem.* **31**, 119–129
- Harm, K. & Hilz, H. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 1469–1479
- Hecht, N. B. (1973) *Biochim. Biophys. Acta* **312**, 471–483
- Hecht, N. B. & Davidson, D. (1973) *Biochem. Biophys. Res. Commun.* **51**, 299–305
- Holmes, A. M. & Johnston, I. R. (1973) *FEBS Lett.* **29**, 1–5
- Holmes, A. M., Hesslewood, I. P. & Johnston, I. R. (1973) *Biochem. Soc. Trans.* **1**, 1134–1135
- Kornberg, A. (1955) *Methods Enzymol.* **1**, 441–443
- Lazarus, L. H. & Kitron, N. (1973) *J. Mol. Biol.* **81**, 529–534
- Loeb, L. A. (1969) *J. Biol. Chem.* **244**, 1672–1681
- Margoliash, E. (1962) *J. Biol. Chem.* **237**, 2161–2174
- Martin, R. G. & Ames, B. N. (1961) *J. Biol. Chem.* **236**, 1372–1379

- Samejima, T. & Yang, J. T. (1963) *J. Biol. Chem.* **238**, 3256–3261
- Sedwick, D. W., Wang, T. S.-F. & Korn, D. (1972) *J. Biol. Chem.* **247**, 5026–5033
- Sumner, J. B. (1955) *Methods Enzymol.* **2**, 378–379
- Tanabe, K. & Takahashi, T. (1973) *Biochem. Biophys. Res. Commun.* **53**, 295–301
- Wallace, P. G. & Hewish, D. R., Venning, M. M. & Burgoyne, L. A. (1971) *Biochem. J.* **125**, 47–51
- Wang, T. S.-F., Sedwick, W. D. & Korn, D. (1974) *J. Biol. Chem.* **249**, 841–850
- Wicha, M. & Stockdale, F. E. (1972) *Biochem. Biophys. Res. Commun.* **48**, 1079–1087