Properties of a terminal deoxyribonucleotidyltransferase isolated from wheat germ

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(Received 12 February 1980/Accepted 2 May 1980)

An enzyme able to catalyse the polymerization of deoxyribonucleotides in a template-independent manner was isolated from dry wheat germ. This activity is associated with a soluble protein which is homogeneous with respect to the molecular weight (approx. 500 000) and, under denaturing conditions, dissociates into product of two size classes, 67 000 and 45 000 daltons respectively. The enzyme-catalysed polymerization can be primed by oligo- as well as poly-deoxyribonucleotides, and is highly efficient (234 nmol/h per mg of finally purified protein) when only one of the four deoxyribonucleoside 5'-triphosphates is present in the incubation mixture. An extension of the 3'-hydroxy termini of polydeoxyribonucleotide chains for approx. 40 nucleotide residues was achieved when non-denatured DNA and [³H]dTTP were used as the primer and substrate respectively. It is concluded that the enzyme isolated from wheat germ shares catalytic properties with the terminal deoxynucleotidyltransferase of mammalian thymus. Unlike that transferase, however, the plant enzyme prefers non-denatured to single-stranded DNA as primer and requires both Mg²⁺ and Mn²⁺ ions for maximal activity.

The only enzyme thus far known to be able to catalyse polydeoxyribonucleotide-chain synthesis without template instructions is terminal deoxynucleotidyltransferase (EC 2.7.7.31). The enzyme has a unique species and tissue distribution. As reviewed by Bollum (1974), it occurs only in mammals and some other vertebrate species. Moreover, its occurrence in these organisms is limited to thymus gland and thymus-derived cells exclusively. Unexpectedly, however, Srivastava (1972) has isolated a terminal-transferase activity from cultured tobacco tissues, and our preliminary data (Brodiewicz-Proba & Buchowicz, 1976) have indicated the occurrence of a similar enzyme activity in germinating wheat embryos. Attempts were therefore made to elucidate whether the wheat-embryo enzyme may be a plant counterpart of the mammalian terminal transferase. It was found that the plant terminal transferase catalyses essentially the same reaction as the calf thymus enzyme does, but differs from it in some catalytic requirements and is associated with a protein of a much higher molecular weight.

Abbreviations used: SDS, sodium dodecyl sulphate; terminal transferase, terminal deoxynucleotidyltransferase (EC 2.7.7.31).

Materials and methods

Plant material

Wheat germ was obtained from a local mill and other commercial sources, including General Mills Inc., Minneapolis, MN, U.S.A. For comparison, embryos isolated from dry grain of wheat (*Triticum aestivum* L. var. Kutnowska) by the method of Johnston & Stern (1957) were also used.

Reagents

[Me-³H]Thymidine 5'-triphosphate and [5-³H]uridine 5'-triphosphate were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Snake-venom and bovine spleen phosphodiesterases were from Worthington Biochemical Corp., Freehold, NJ, U.S.A. Chemicals were supplied by the companies listed: Sephadex G-25 and Sepharose 6B, Pharmacia (Uppsala, Sweden); DEAE-cellulose (DE-52) and phosphocellulose (P11), Whatman (Maidstone, Kent, U.K.); calf thymus DNA, unlabelled nucleoside triphosphates and *N*-ethylmaleimide, Sigma (St. Louis, MO, U.S.A.); oligothymidylic acid [d(pT)₁₀], Calbiochem (Los Angeles, CA, U.S.A.); electrophoresis reagents, apoferritin, ovalbumin and bovine serum albumin, Serva Feinbiochemica (Heidelberg, Germany). All other com-

pounds were obtained commercially and were of reagent-grade quality.

Purification procedure

A sample (17 g) of dry commercial wheat germ was ground with a pestle and mortar in buffer A (100 mM-Tris/HCl, pH 7.9, 5 mM-magnesium acetate, 1 mM-dithiothreitol, 0.05 mM-phenylmethanesulphonyl fluoride and 250 mM-sucrose). The homogenization and all the subsequent operations were carried out at 4°C. The slurry was filtered through four layers of gauze and the filtrate (approx. 100 ml) centrifuged at 23 000 g for 20 min (step 1). Solid $(\text{NH}_4)_2\text{SO}_4$ was added to the supernatant and the precipitate formed between 33 and 66% saturation with the salt was collected by centrifugation for 20 min at 15 000 g. The $(\text{NH}_4)_2\text{SO}_4$ fraction was dissolved in 15 ml of buffer A (step 2) and loaded on a Sephadex G-25 column (4 cm $\times$ 20 cm). The effluent corresponding to the first protein peak (40 ml) was kept (step 3). The Sephadex G-25 fraction was concentrated by precipitation with 70%-satd. $(\text{NH}_4)_2\text{SO}_4$ and, after being dissolved in buffer B (50 mM-Tris/HCl, pH 7.9, 1 mM-dithiothreitol and 200 mM-sucrose), was applied to a Sepharose 6B column (1.6 cm $\times$ 90 cm) and eluted with the same buffer. Fractions containing nucleoside triphosphate-inhibited activity (see Fig. 1) were pooled (step 4) and applied to a DEAE-cellulose DE-52 column (1 cm $\times$ 14 cm). The column was washed with buffer B and the adsorbed material eluted with a salt step (20 ml of 10 mM-KCl followed by 20 ml of 100 mM-KCl, both in buffer B). Fractions (1.1 ml) were collected, and those corresponding to the peak of enzyme activity (fractions 24–29) were pooled (step 5). The DE-52 fraction was loaded on a phosphocellulose P11 column (0.5 cm $\times$ 4 cm), which was previously equilibrated with buffer B plus 100 mM-KCl. The column was washed with 5 ml of the same solution, followed by 10 ml of 160 mM-KCl in buffer B. After these washings, the enzyme was eluted with buffer B containing 450 mM-KCl and fractions (0.9 ml) were collected. The second of them was found to contain most of the enzyme activity, and this was used as the finally purified enzyme preparation (step 6). The preparation was stored at –20°C with a negligible loss of the activity within several weeks. A summary of the purification steps is shown in Table 1.

Standard assay conditions

The standard incubation mixture of 0.1 ml contained 15 mM-Tris/HCl, pH 7.9, 150 mM-KCl, 5 mM-dithiothreitol, 5 mM-magnesium acetate, 0.6 mM-MnCl₂, 1 mM-[³H]dTTP of low specific radioactivity (25 Ci/mol), 30 μ g of highly polymerized calf thymus DNA, and enzyme (usually approx. 10 μ g of protein). In some auxiliary experiments (see below),

d(pT)₁₀ was used instead of DNA. After incubation at 37°C for 30 min, the reaction was stopped and radioactivity of the product was measured similarly to the method of Coleman (1977). Briefly, a sample (90 μ l) of the reaction mixture was spotted on a strip of Whatman 3MM chromatography paper on which non-labelled dTTP had been spotted at the origin. The strip was developed for approx. 6 h in a mixture of 96% (v/v) ethanol and 1 M-ammonium acetate (1:1, v/v). After drying, the origin area of the strip was cut out and counted for radioactivity in 5 ml of toluene containing 2,5-diphenyloxazole (0.5%, w/v) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (0.03%, w/v).

One unit of the enzyme activity represents 1 nmol of nucleotide polymerized in 1 h.

Tests for interfering enzyme activities

Assays for DNA-dependent DNA polymerases were made as described by Castroviejo *et al.* (1979). RNA polymerases I and II were assayed under conditions described previously (Mazuś & Brodniewicz-Proba, 1976). Nuclease activity was measured as described by Okamura *et al.* (1978), with calf thymus DNA as the substrate.

Polyacrylamide-slab-gel electrophoresis

The purified enzyme preparation was analysed by 7.5%-polyacrylamide-slab-gel electrophoresis both under non-denaturing conditions and in the presence of 0.1% SDS. Gels were stained for protein with Coomassie Brilliant Blue R-250 and destained in acetic acid. Buffers and solutions were prepared as described by Laemmli (1970).

Isolation of the enzyme-catalysed-reaction product and its further treatments

The oligo(dT)-primed-reaction product was obtained under conditions similar to those described for the standard assay, except that DNA was replaced by 1 μ g of d(pT)₁₀ and incubation was for 1 h. At the end of incubation, a further 10 μ g of the oligonucleotide and 10 μ mol of non-labelled dTTP were added, and the mixture was chromatographed in the same solvent system as described above (see under 'Standard assay conditions'). The origin area of the chromatogram was cut out and eluted with water. The water eluates of ten parallel experiments were pooled, concentrated under reduced pressure, applied to a 10%-polyacrylamide gel and electrophoresed under conditions described by Loening (1967). Cylindrical (1 cm $\times$ 11 cm) gels were used, and the buffer contained 36 mM-Tris, 30 mM-NaH₂PO₄, 1 mM-EDTA, and 0.2% SDS, pH 7.8. Electrophoresis was at a constant voltage of 50 V and with a current of approx. 5 mA/gel. The gels were scanned in an ISCO absorbance monitor, frozen at –20°C and cut into 2-mm slices. After

solubilization in 30% (w/w) H_2O_2 , the radioactivity of each slice was measured by using Bray's (1960) scintillator and a Packard liquid-scintillation counter.

The DNA-primed-reaction product was obtained under standard assay conditions, but with a 10-fold larger incubation mixture. At the end of incubation, 1 mg of DNA and 100 μmol of non-labelled dTTP were added and the mixture was treated with equal volume of 10% (w/v) trichloroacetic acid at 0°C. The precipitate formed was collected by centrifugation for 10 min at 18000g, washed twice with 5% trichloroacetic acid and dissolved in 5 ml of a medium containing 150 mM-NaCl, 15 mM-trisodium citrate and 0.1 mM-EDTA, pH 7.0. The solution was then treated with 2 vol. of 96% (v/v) ethanol and left at 0°C for 1 h. The resulting precipitate was again collected by centrifugation (23000g, 20 min), washed three times with 70% ethanol, once with 99.8% ethanol, and desiccated. The dry product was subjected either to a mild acid hydrolysis or phosphodiesterase treatments.

The acid hydrolysis was carried out under conditions (Shapiro & Chargaff, 1963) leading to the release of pyrimidine oligonucleotides. A sample of the product, usually 1 mg, was dissolved in 0.13 ml of 0.1 M- H_2SO_4 and hydrolysed for 35 min at 100°C in a sealed ampoule. The hydrolysate, in 10–20 μl portions, was applied to 10%-polyacrylamide gels and electrophoresed as above.

For the phosphodiesterase treatments, a similar portion of the DNA-primed-reaction product was first pretreated with acid phosphatase (50 $\mu\text{g}/\text{ml}$, 37°C, 15 min) and, after thermal inactivation of the enzyme, recovered by ethanol precipitation. The precipitate was then digested with either snake-venom or bovine spleen phosphodiesterase under conditions similar to those described by Razzell (1963). Incubation mixtures (0.1 ml) containing the reaction product (in amount corresponding to 200 μg of DNA) and either 0.8 unit [one unit hydrolyses 1 μmol of bis(*p*-nitrophenyl) phosphate/min] of

snake-venom phosphodiesterase or 1.2 units of the bovine spleen enzyme were kept at 37°C for 30 min. Radioactivity of the acid-precipitable residue was then determined for each incubation by the filter-paper-disc method of Mans & Novelli (1961).

Protein determination

Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

Purification

Table 1 shows the successive steps involved in the purification of terminal-transferase activity from wheat germ (essentially the same results were obtained independently of the germ source). The first three steps resulted in a poor purification. They were, however, necessary to remove starch-like material and pigments, which are abundant in plant tissues. The purification step 4 was of crucial importance. As shown in Fig. 1, it resulted in the separation of nucleoside triphosphate-inhibited activity from nucleoside triphosphate-stimulated activity. The former seemed to correspond to template-independent DNA polymerase and was kept. The latter, corresponding probably to DNA polymerase- α , was discarded. Thus the interfering DNA-dependent DNA polymerase activity was eliminated at this purification stage, and the efficiency of the further steps can be calculated from the data of Table 1 without special precautions. The overall purification (350-fold) was, however, probably underestimated, as DNA-dependent polymerases could interfere with the estimation of the crude-extract specific activity. Difficulties in the assessment of the crude-extract activity are usually encountered in the purification of terminal-transferase-type enzymes (see, e.g., Siddiqui & Srivastava, 1978). Specific activity of the finally purified preparation (234 units/mg) is far from that

Table 1. *Summary of purification of terminal-transferase activity from wheat germ*

A 17g sample of dry wheat germ was used. For experimental details, see the Materials and methods section, where the unit of the enzyme activity is also defined. The interfering template-dependent activity was eliminated at step 4, which is additionally shown in Fig. 1.

Step	Volume (ml)	Protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)
1. Crude extract	100	3500	2310	0.66	100
2. 33–66%-satd. $(\text{NH}_4)_2\text{SO}_4$	15	1960	1760	0.90	76
3. Sephadex G-25	40	1700	1650	0.97	71
4. Sepharose 6B	16	498	1330	2.68	58
5. DE-52	6.6	4.0	436	109	19
6. Phosphocellulose	0.9	0.6	140	234	6

Fig. 1. Analysis of step 4 (gel filtration on Sepharose 6B) Enzyme from step 3 (see Table 1) was applied to a Sepharose 6B column and the effluent was tested for the enzyme activity under standard conditions (O) and, in parallel, in assays supplemented with $1\text{ }\mu\text{mol}$ of dATP, dCTP and dGTP (●). Absorbance (Δ) was measured with a Varian Techtron spectrophotometer; for other details see the Materials and methods section. V_0 , void volume. The horizontal bar marks the fractions pooled for further purification.

Fig. 2. Sepharose 6B gel filtration of the finally purified enzyme preparation

The enzyme preparation (0.4 mg of protein; specific activity 230 units/mg) was applied to the Sepharose column and eluted under step-4 conditions (see the Materials and methods section). O, Enzyme activity; Δ , protein concentration. The arrows indicate, from left to right, the positions of Blue Dextran (200000 daltons), apoferritin (480000) and bovine serum albumin (67000 daltons) found in a parallel experiment.

of homogeneous calf thymus terminal transferase (Chang & Bollum, 1971), but approaches values reported for highly purified DNA polymerases of plant origin (Castroviejo *et al.*, 1979).

The finally purified preparation was once more gel-filtered through the Sepharose 6B column (Fig. 2). No additional purification was achieved, and the enzyme activity was recovered within the same portion of the effluent as that found in purification step 4 (compare Figs. 1 and 2). Both enzyme activity and protein were eluted as single peaks, perfectly corresponding to each other (see Fig. 2), as might be expected for a homogeneous enzyme preparation. Apparently the terminal-transferase activity is associated with a homogeneous (in respect to the molecular size) protein component of high mol.wt. (approx. 500000). Similarly, when the preparation was subjected to polyacrylamide-gel electrophoresis under non-denaturing conditions, all the protein remained at the starting zone (results not shown). Under denaturing conditions, however, two sets of bands appeared (Fig. 3). Their electrophoretic mobilities corresponded roughly to those found for the 67000- and 45000-dalton markers respectively. Probably the undissociated enzyme preparation was composed of several subunits of each size class.

Neither DNA nor RNA components could be detected in the purified enzyme preparation by its hydrolysis with conc. HClO_4 followed by paper chromatography in a water-saturated butan-1-ol solvent system (Wyatt, 1955).

Catalytic requirements

The requirements of the isolated enzyme are summarized in Table 2. The omission of DNA from the standard incubation mixture drastically decreased the observed $[^3\text{H}]\text{dTTP}$ incorporation. The DNA could, however, be replaced by oligo(dT), indicating that the polydeoxyribonucleotide serves as primer and not as template. Also, in a manner typical for template-independent polymerases, the presence of other nucleoside triphosphates considerably inhibited the labelled dTTP incorporation. Noticeably, non-denatured DNA had a higher priming efficiency than the heated one. RNA preparations were poor primers. Both Mg^{2+} and Mn^{2+} ions, as well as a relatively high KCl concentration, were necessary for the maximal enzyme activity. Unlike calf thymus terminal transferase, the plant enzyme did not prefer cacodylate to the Tris/HCl buffer. The reaction was strongly inhibited by *N*-ethylmaleimide (an inhibitor for both terminal transferase and DNA polymerase- α , but

Fig. 3. SDS/polyacrylamide-slab-gel electrophoresis of the finally purified enzyme preparations

Gels: 1 and 2, 15 μ g portions of the enzyme preparations purified to step 6; 3, a 10 μ g portion of the enzyme preparation purified to step 6 and additionally gel-filtered on Sepharose 6B; 4 and 5, reference samples of hen ovalbumin (45000 daltons) and bovine serum albumin (67000 daltons) respectively. The origins are at the top; narrow bands at the bottom correspond to the migration front.

not for DNA polymerase- β). The reaction rate was maximal at pH 7.9 and declined sharply above pH 8.0 (results not shown). The enzyme activity was linear with respect to time (up to 60 min) and protein concentration (within the range 2–20 μ g/0.1 ml).

Under conditions optimal for DNA-dependent DNA polymerases the enzyme preparation had a low activity, incorporating less than 10 nmol of dTTP/mg of protein in 1 h. [3 H]UTP was incorporated (under standard conditions) at a rate corresponding to 5% of that found for the [3 H]dTTP incorporation. The observed [3 H]UTP incorporation was not influenced by α -amanitin (1 μ g/ml) and could not be improved by changing the incubation conditions to those optimal for RNA polymerases. It is therefore concluded that the isolated preparation was contaminated neither with DNA-dependent DNA polymerases nor with RNA polymerases. There was also no DNA-degrading activity associated with the purified enzyme preparation.

By assuming the molecular weight of the non-denatured DNA used as 10^6 or more (as assessed tentatively by Sepharose 6B gel-filtration), it may be calculated from the data of Table 2 that at least 15 thymidylate residues per polydeoxyribonucleotide chain were attached. For the d(pT) $_{10}$ -primed reaction, the average chain addition was manyfold lower. Probably there was an excess of the primer molecules when DNA was replaced by an equal amount (in weight units) of the oligonucleotide. Therefore the amount of d(pT) $_{10}$ was considerably decreased when it was aimed to establish the maximal size of the reaction product formed (see the next paragraph).

Table 2. Requirements of the isolated enzyme preparation

The finally purified preparation was incubated under standard assay conditions (see the Materials and methods section) with the indicated omissions from, and additions to, the standard reaction mixture (0.1 ml). 3 dNTP stands for a mixture of dATP, dCTP and dGTP.

Omissions	Additions	dTMP incorporated (nmol/assay)	Relative activity (%)
None	None	1.26	100
DNA	None	0.07	6
DNA	Heated DNA (30 μ g)	0.38	30
DNA	d(pT) $_{10}$ (30 μ g)	1.29	102
DNA	Wheat-germ total RNA (30 μ g)	0.14	11
DNA	Yeast tRNA (30 μ g)	0.16	13
None	3 dNTP (1 μ mol of each)	0.13	10
None	3 dNTP (0.1 μ mol of each)	0.50	40
Mg $^{2+}$, Mn $^{2+}$	None	0.15	12
Mg $^{2+}$	None	0.57	45
Mn $^{2+}$	None	0.33	26
Mg $^{2+}$, Mn $^{2+}$	CoCl $_2$ (0.2 μ mol)	0.52	41
KCl	KCl (5 μ mol)	0.50	40
Tris/HCl	Cacodylate (5 μ mol)	0.77	61
None	N-Ethylmaleimide (1 μ mol)	0.07	6
Enzyme	None	<0.02	<2

Characterization of the enzyme-catalysed-reaction product

Under standard incubation conditions, [^3H]dTTP was incorporated into a product that could be separated from the radioactive precursor by paper chromatography as well as by precipitation with trichloroacetic acid and ethanol (see the Materials and methods section). The radioactive high-molecular-weight product resisted Pronase (0.1 mg/ml, 30°C, 30 min) and alkaline (0.3 M-KOH, 37°C, 2 h) treatments, but was degraded under mild acid-hydrolysis conditions (see below).

When d(pT) $_{10}$ was used as primer, radioactive products differing in size within a narrow range appeared. Most of them moved somewhat slower than the internal d(pT) $_{10}$ marker when electrophoresed on a 10%-polyacrylamide gel (Fig. 4). Evidently, on incubation with the enzyme and [^3H]dTTP, the primer molecules were extended by several thymidylate residues.

The DNA-primed reaction product did not move under the same electrophoresis conditions. Upon hydrolysis (0.1 M-H $_2$ SO $_4$, 100°C, 35 min), however, radioactive polypyrimidine blocks of various size appeared. The most frequent of them corresponded

in size to products of approx. 40 nucleotides in length (Fig. 4). The attached [^3H]thymidylate residues could be removed from the DNA primer by digestion with snake-venom, but not bovine spleen, phosphodiesterase (approx. 3 and 99% of the initial radioactivity remained acid-insoluble after these treatments respectively). It is therefore assumed that the isolated enzyme extends the 3'-hydroxy end of the polydeoxyribonucleotide chain for a series of nucleotide units and may be used for DNA tailing.

Both the catalytic requirements and the nature of the reaction product formed are consistent with the assumption that the isolated enzyme preparation contains a terminal deoxynucleotidyltransferase activity.

Discussion

The isolated terminal-transferase activity is associated with a protein whose molecular weight is much higher than that of any other deoxyribonucleotide-polymerizing enzymes (cf. data quoted by Sarngadharan *et al.*, 1978). With respect to its catalytic activity, the obtained preparation is, however, similar to the mammalian terminal transferase (Chang & Bollum, 1971; Bollum *et al.*, 1974). Both enzymes extend the 3'-hydroxy end of polydeoxyribonucleotide chains for a series of nucleotide residues in a template-independent manner. Their catalytic requirements are also generally similar. Nevertheless, their responses to bivalent cations and pH, buffer and salt concentration changes (compare the data of Table 2 with those given by Bollum *et al.*, 1974) differ significantly. Also, unlike calf thymus terminal transferase, the plant enzyme prefers non-denatured to single-stranded DNA as primer. In this respect, the wheat-germ preparation resembles that isolated by Srivastava (1972) from cultured tobacco tissues. However, unlike the tobacco terminal transferase, the wheat enzyme does not seem to be associated with chromatin and can be easily isolated from the post-mitochondrial-supernatant fraction.

Apparently the isolated enzyme preparation represents a new example of a template-independent deoxyribonucleotide-polymerizing activity. It cannot, however, be excluded that the preparation corresponds to an enzyme whose true template is at present unknown. On the other hand, it seems rather unlikely that the isolated activity could arise as an artefact. A limited proteolysis of DNA-directed DNA polymerases, as suggested by Johnson & Morgan (1976) for mammalian terminal-transferase activity appearance, could not result in the formation of a product of such a high molecular weight (approx. 500 000). Similarly, an unspecific aggregation of smaller products also does not seem to be the case, since the activity remained associated with

Fig. 4. Polyacrylamide-gel-electrophoretic analysis of the enzyme-catalysed reaction products

The d(pT) $_{10}$ -primed-reaction product (total radioactivity 6000 d.p.m.) was isolated and directly applied to the gel as described in the Materials and methods section under 'Isolation of the enzyme-catalysed reaction product and its further treatments'. The DNA-primed-reaction product (total radioactivity 9000 d.p.m.) was first hydrolysed (0.1 M-H $_2$ SO $_4$, 100°C, 35 min) and then applied to a parallel gel. Radioactivity is given as d.p.m./2 mm slice. O, d(pT) $_{10}$ -primed-reaction product; ●, acid hydrolysate of the DNA-primed-reaction product. Electrophoresis was from left to right, and the arrows indicate the positions of tRNA (left) and d(pT) $_{10}$ (right).

the high-molecular-weight protein component after a series of column chromatographies (see Figs. 1 and 2).

Deibel & Coleman (1979) have purified a high-molecular-weight terminal transferase from human lymphoblasts. A structural relationship between the human and plant terminal transferases may be envisaged, as both enzymes have similar SDS/polyacrylamide-gel-electrophoresis patterns. The increasing number of reports on the isolation of terminal-transferase-type enzymes from non-thymus sources seems to indicate that the ability to synthesize polydeoxyribonucleotide chains without template instruction may be a common property for a variety of eukaryotic cells.

This work was supported by the Polish Academy of Sciences within the project 09.7.

References

- Bollum, F. J. (1974) *Enzymes 3rd Ed.* **10**, 145–171
- Bollum, F. J., Chang, L. M. S., Tsiapalis, C. M. & Dorson, J. W. (1974) *Methods Enzymol.* **29**, 70–81
- Bray, G. A. (1960) *Anal. Biochem.* **1**, 279–285
- Brodniewicz-Proba, T. & Buchowicz, J. (1976) *FEBS Lett.* **65**, 183–186
- Castroviejo, M., Tharaud, D., Tarrago-Litvak, L. & Litvak, S. (1979) *Biochem. J.* **181**, 183–191
- Chang, L. M. S. & Bollum, F. J. (1971) *J. Biol. Chem.* **246**, 909–916
- Coleman, M. S. (1977) *Nucleic Acids Res.* **4**, 4305–4312
- Deibel, M. R. & Coleman, M. S. (1979) *J. Biol. Chem.* **254**, 8634–8640
- Johnson, D. & Morgan, A. R. (1976) *Biochem. Biophys. Res. Commun.* **72**, 840–849
- Johnston, F. B. & Stern, H. (1957) *Nature (London)* **179**, 160–161
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Loening, U. E. (1967) *Biochem. J.* **102**, 251–257
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mans, R. J. & Novelli, G. D. (1961) *Arch. Biochem. Biophys.* **94**, 48–53
- Mazùs, B. & Brodniewicz-Proba, T. (1976) *Acta Biochim. Pol.* **23**, 261–267
- Okamura, S., Crane, F., Messner, H. A. & Mak, T. W. (1978) *J. Biol. Chem.* **253**, 3765–3767
- Razzell, W. E. (1963) *Methods Enzymol.* **6**, 236–258
- Sarnagadharan, M. G., Robert-Guroff, M. & Gallo, R. C. (1978) *Biochim. Biophys. Acta* **516**, 419–487
- Shapiro, H. S. & Chargaff, E. (1963) *Biochim. Biophys. Acta* **76**, 1–8
- Siddiqui, F. A. & Srivastava, B. I. S. (1978) *Biochim. Biophys. Acta* **517**, 150–157
- Srivastava, B. I. S. (1972) *Biochem. Biophys. Res. Commun.* **48**, 270–273
- Wyatt, G. R. (1955) in *The Nucleic Acids* (Chargaff, E. & Davidson, J. N., eds.), vol. 1, pp. 243–265, Academic Press, New York