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MOLECULAR WEIGHTS OF THE MAJOR DNA POLYMERASES IN A HIGHER PLANT, PISUM SATIVUM L. (PEA)

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The molecular weights of the soluble (α-like) and chromatin-bound (β-like) DNA polymerases of pea have been determined. The bulk of the soluble activity consists of molecular species of ca 101,500 and ca 140,000 molecular weights. Smaller (49,000) and larger (182,000 and 234,000) species are also observed in some preparations. The chromatin-bound DNA polymerase exhibits a molecular weight of ca 50,000, although a larger (ca 88,000) species is also detected under conditions which favour aggregate formation.

There is at present some confusion concerning the nature of the DNA polymerases of higher plants (reviewed in ref. 1). Some investigators have suggested that higher plant DNA polymerases resemble those of lower eukaryotes and that in particular, plants do not possess a low-molecular-weight, chromatin-bound DNA polymerase of direct equivalence to the polymerase-β of vertebrates (2,3,4). Others have suggested that enzymes directly equivalent to the polymerases α, β and γ of vertebrates are present in higher plants (1,5,6,7,8). It is certainly clear that higher plants contain an enzyme with very similar properties to polymerase-α (1). In this laboratory we have characterised this soluble α-like enzyme in pea, showing it to be sensitive to KCl, N-ethyl-maleimide, phosphonoacetate (5,9) and aphidicolin (10), and to be the major polymerase activity in dividing cells (11). We have also detected in pea a completely different DNA polymerase, which is tightly bound to the chromatin, which is stimulated by KCl (5) and which does not show a correlation with cell division activity (11). It is not inhibited by aphidicolin (10). However, unlike the vertebrate polymerase-β, it is inhibited by phosphono-

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acetate and N-ethyl maleimide, but much less so than the soluble α-like enzyme (5). This chromatin-bound DNA polymerase in pea thus has a number of distinct similarities to the DNA polymerase-β of vertebrates, although there are minor differences in responses to inhibitors.

Both the soluble (α-like) polymerase and the chromatin-bound (β-like) polymerase of pea exhibit heterogeneity when chromatographed on DEAE-Sephadex (5,9). For the soluble enzyme, the bulk of the activity elutes as a large complex peak consisting of at least two molecular species. A minor species, less sensitive to N-ethyl-maleimide than the major species, is seen in preparations which are subjected freeze-thaw prior to chromatography. The chromatin-bound enzyme elutes as two clear-cut peaks, the major one containing ca 80% of the total activity.

At the time of publication of these characterisations of the soluble, α-like and chromatin-bound, β-like polymerases of pea, we were prevented by technical problems from determining the size of the enzymes. These problems (which are summarised in the results section) have been overcome to the extent that we can now present estimates of molecular weight for both enzymes.

MATERIALS AND METHODS

Plant material. Pea seeds (Pisum sativum L., cv Feltham First) were germinated and grown in darkness at 25°C. After five days, the shoot apices were harvested and used for extraction of soluble and chromatin-bound DNA polymerases.

Enzyme extraction and partial purification. Methods for enzyme extraction and partial purification have been described in detail elsewhere (5,9), but are briefly summarised here. For the soluble polymerase, 34,000 x g or 100,000 x g supernatants of tissue homogenates were used as crude enzyme preparations. Partial purification was achieved by $(NH_4)_2SO_4$ fractionation, de-salting through Sephadex G-50 and chromatography on DEAE-cellulose or DEAE-Sephadex. For the chromatin-bound enzyme, chromatin preparations were used as a source of the enzyme. Partial purification was achieved by solubilizing the enzyme with 2M $(NH_4)_2SO_4$ followed by de-salting through Sephadex G-50 (the de-salted preparation being taken as 'crude' enzyme) and then chromatography on DEAE-cellulose or DEAE-Sephadex.

Enzyme assays were carried out as described previously (5,9).

Molecular weight determinations

(i) Gel filtration was carried out on columns (450 x 25 mm) of Sepharose-6B, using Dextran Blue, haemoglobin, alkaline phosphatase, myoglobin and a set of ^{14}C-marker proteins as standards. Fractions of 2.5 mls column eluate

were collected automatically and each fraction was assayed for DNA polymerase activity and for the presence of standards.

(ii) Centrifugation in glycerol density gradients, using alkaline phosphatase as a standard, was carried out as described by McLennan and Keir (12). Centrifugation time was varied between 10 and 18 h according to the enzyme under investigation. After centrifugation, tube contents were expelled by upward displacement; fractions of 250 µl were collected and assayed for DNA polymerase and alkaline phosphatase activities.

RESULTS

Partially purified enzyme preparations. Ideally, the partially purified enzyme preparations obtained by ion-exchange chromatography should have been used for molecular weight determinations. However, although such preparations are stable when stored at -20°C (5,9), both the soluble and the chromatin-bound enzymes lost a very high proportion of their activity when subjected to gel filtration or to density gradient centrifugation. Very large amounts of activity needed to be loaded in order to obtain fractions active enough to assay reliably, and for the chromatin-bound enzyme in particular, it proved difficult to obtain partially purified preparations which were active enough to be used in this way. Despite these problems, values in the range 100,000 to 150,000 were obtained by gel filtration for the major population of the soluble enzyme.

Crude preparations

(i) Soluble, α-like polymerase. For the soluble DNA polymerase, aliquots of 100,000 x g supernatants of tissue homogenates were fractionated by gel filtration or by density gradient centrifugation. In gel filtration experiments, a large proportion of the activity bound to the Dextran Blue (used for determination of Vo). The remainder exhibited a very high apparent molecular weight. Dextran Blue was therefore omitted from the samples, and Vo was determined in separate elutions from those used to fractionate the enzyme. Under these conditions, the majority of the soluble DNA polymerase activity eluted as a somewhat heterogeneous peak in the molecular weight range 110,000 to 150,000.

Glycerol density gradients proved to be routinely successful in giving reproducible fractionations of the crude soluble polymerase. Gradients were

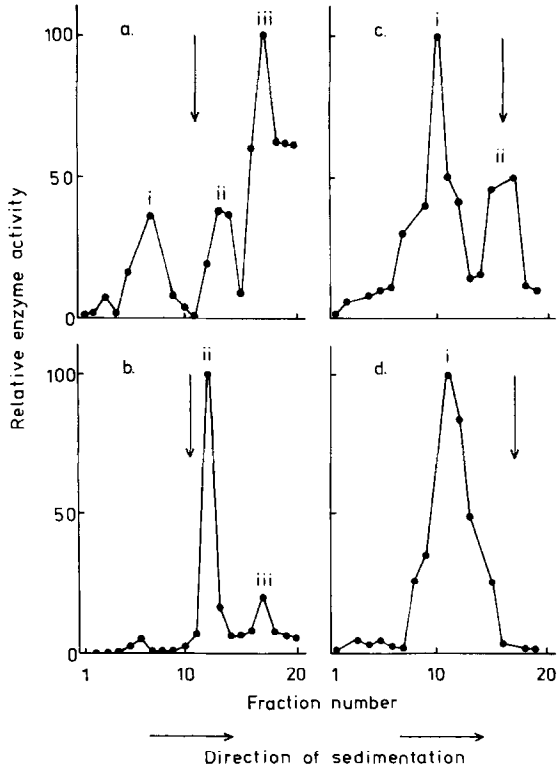

Figure 1. Glycerol density gradient centrifugation of DNA polymerases from pea. 1a and 1b: soluble DNA polymerase. 1c and 1d: chromatin-bound DNA polymerase. In 1a and 1c, KCl was not present during centrifugation; in 1b and 1d, IM KCl was present during centrifugation. The vertical arrows indicate the positions of the alkaline phosphatase marker. Data are expressed as relative enzyme activities, in order to be able to directly compare different enzyme preparations. For the soluble enzyme, 100 represents an activity of between 1.8 and 2.7 pmol d-TMP/fraction/minute and for the chromatin-bound enzyme 100 represents an activity of between 0.7 and 0.9 pmol d-TMP/fraction/minute.

run in the presence of KCl (IM) or in its absence. Representative gradients are shown in Fig. 1, a & b and the data from all experiments are summarised in Table 1. In all the fractionations, in the presence or absence of KCl, the two peaks designated ii and iii were present, although their proportions relative to each other varied. The variation in the relative proportions of the two peaks was not related to the presence or absence of KCl. Peak ii had a molecular weight of ca 101,500 whilst peak iii had a molecular weight of 136,000 to 144,000. In some of the fractionations where KCl was absent, another peak (peak i) was present; this had a molecular weight of ca 49,000.

Table 1 Molecular weights of soluble DNA polymerase determined by glycerol density gradient centrifugation. Figures in brackets indicate the number of determinations, and ± values indicate SEM, except where n is less than 3, when ± values indicate range.

Peak	Molecular Weight	
	KCl present	KCl absent
i	-	49,300 ± 7,300 (3)
ii	101,400 ± 9,200 (5)	101,600 ± 3,200 (5)
iii	143,800 ± 3,700 (5)	135,800 ± 5,800 (5)
iv	182,100 ± 9,400 (3)	-
v	234,000 ± 7,700 (2)	-

In some of the fractionations where KCl was present, two larger molecular species (iv and v) were observed. Relatively short centrifugation times were necessary for good resolution of these larger peaks, and under such conditions they exhibited molecular weights of ca 182,000 and 234,000.

(ii) Chromatin-bound, ß-like polymerase. As with the soluble enzyme, centrifugation in glycerol density gradients gave reproducible fractionations of the chromatin-bound polymerase. Centrifugation was carried out with the solubilized, de-salted enzyme in the presence of KCl (IM) or in its absence. Representative fractionations are shown in Fig 1 c & d, and all the data are summarised in Table 2. In all experiments, in the presence or absence of KCl, a major peak (peak i), with a molecular weight of 47,000 to 54,000, was

Table 2 Molecular weights of chromatin-bound DNA polymerase determined by glycerol densely gradient centrifugation. Figures in brackets indicate the number of determination; ± values indicate SEM, except where n is less than 3, when ± values indicate range.

Peak	Molecular Weight	
	KCl present	KCl absent
i	46,700 ± 2,900 (4)	53,400 ± 7,200 (3)
ii	-	88,000 ± 600 (2)

obtained. In two of the three experiments in which KCl was absent, a second, larger molecular species (peak ii), with a molecular weight of ca 88,000, was also observed.

DISCUSSION

In determining the molecular weights of the pea DNA polymerases, two problems have been encountered. The reason for the lack of stability of the partially purified enzymes during gel filtration or density gradient centrifugation is not known. A similar problem has been reported for DNA polymerases from several other plants (13), although partially purified DNA polymerases from at least three plants, wheat (3), perwinkle (4) and beet (6) have been successfully subjected to gel filtration and/or gradient centrifugation. The second problem, namely the binding of the soluble DNA polymerase to Dextran Blue, has also been reported for some mammalian DNA polymerases (14) and indicates that gel filtration data for DNA polymerases must be interpreted with care.

In our experiments with crude preparations of the soluble DNA polymerase, the major proportion of the activity was clearly a heterogeneous population with peaks showing molecular weights of 101,500 and 136,000 to 144,000. The size of the larger of these two peaks is very similar to that of the catalytic sub-unit of DNA polymerase-α from rat (15), calf-thymus (16,17) and Drosophila (15,16). In comparison with the data for the calf-thymus enzyme, the peak at 101,5000 in our experiments is regarded as being a partial proteolysis product of the catalytic sub-unit. The peak at 49,000, observed in only a small number of experiments, is also regarded as a proteolysis product of the catalytic sub-unit, similar to the 70,000 molecular weight species seen in Drosophila polymerase-α (16) and the 70,000 and 35,000 molecular weight species seen in some calf-thymus polymerase-α preparations (16). It must be emphasised, in view of the data we obtained for the chromatin-bound enzyme, that this fraction of the soluble enzyme was not caused by contamination with traces of the chromatin-bound enzyme, since

firstly, the initial preparation methods for the two enzymes gave no measurable cross-contamination, and secondly, the assay conditions used for the soluble polymerase were not suitable for the chromatin-bound enzyme.

The largest molecular weight species of the soluble polymerase observed in our experiments (182,000 and 234,000) were only observed when gradients were run in the presence of IM KCl. It is therefore unlikely (although not impossible) that these were random aggregates. In view of data concerning the DNA polymerase-α of a number of other organisms (15,17) it is most likely that these larger molecular species represented associations of the catalytic sub-unit with one or more of the non-catalytic sub-units thought to be present in the native enzyme.

In comparison with our previous data, (see Introduction), it is likely that the major, somewhat heterogeneous peak eluted from the ion-exchange columns in our earlier experiments was a mixed population of the higher molecular weight species (i.e. 101,000 upwards), whilst the minor peak, less sensitive to N-ethyl maleimide, was the 49,000 molecular weight species (cf 18).

The data for the chromatin-bound DNA polymerase of pea are very similar to those obtained for the polymerase-β of animal cells (19). The molecular weight of the single molecular species observed in gradients run in the presence of KCl (ca 50,000), and the appearance of a second molecular species, with a molecular weight ca twice that of the first molecular species (allowing for the uncertainties inherent in estimations of molecular weight by preparative scale density gradient centrifugation) are indications that the chromatin-bound polymerase is probably a small protein which is liable to form aggregates in the absence of salts (cf ref. 20). Comparison with our data obtained earlier by DEAE-sephadex chromatography (see Introduction) suggests that the major peak eluting from the ion-exchange columns was the lower molecular weight species, whilst the minor peak was the putative aggregate.

Overall, then, the data presented in this paper support our earlier contention that plants contain DNA polymerases of direct equivalence to polymerase-α and polymerase-β of animals. Recent work has also indicated the presence of a polymerase-γ-like enzyme in plants (1,8,13). In fact, of all the multicellular organisms investigated, only fungi and slime-moulds do not appear to possess the range of polymerases known as α, β and γ.

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