A low molecular weight DNA polymerase from wheat embryos

Michel Castroviejo, Marie-Thérèse Gatius and Simon Litvak*
Institut de Biochimie Cellulaire et Neurochimie du CNRS, 1 rue Camille Saint Saëns, 33077 Bordeaux Cedex, France (*author for correspondence); 1 Present address: INRA-Laboratoire L.M.T.C., BP 527, 44026 Nantes Cedex 03, France

Received 13 December 1989; accepted in revised form 29 May 1990

Key words: wheat embryos, low molecular weight DNA polymerase

Abstract

The study of plant DNA polymerases lags far behind that concerning their animal or yeast counterpart. In this work we describe the first extensive purification to apparent homogeneity, as well as a detailed biochemical and immunological characterization, of a low molecular weight DNA polymerase (DNA polymerase C1) purified from wheat embryos. The monomeric enzyme is a basic protein having a molecular weight of 52 kDa. Polyclonal antibodies raised in rabbits against DNA polymerase C1 did not inhibit animal DNA polymerases α and β or wheat DNA polymerase A, whereas wheat DNA polymerases C11 and B were much less affected than the C1 enzyme. Several properties of enzyme C1 were studied. Some known inhibitors of DNA polymerase activity including aphidicolin, phosphonoacetic acid and heparin, did not affect DNA polymerase C1 while the activity of this enzyme was strongly inhibited by ddTTP and N-ethylmaleimide. The polyamine spermine decreased markedly the enzyme activity, while spermidine produced a strong stimulation at the same concentrations that spermine inhibited the enzyme. The best template for this enzyme is poly dA-oligo dT, although polymerase C1 can recognize significantly some synthetic polynucleotide templates (poly rC-oligo dG, poly rA-oligo dT) but only at a given protein/template primer ratio. The enzyme is blocked at the amino terminus, thus preventing the automatic sequencing of the protein. The amino acid analysis showed a striking similarity with the animal low molecular weight DNA polymerase β. The latter observation, as well as the effect of inhibitors (except N-ethylmaleimide which does not inhibit the animal polymerase) indicate that the DNA polymerase described in this work is a plant DNA polymerase very similar to the low molecular weight animal DNA polymerase β, an enzyme believed to be involved in nuclear DNA repair.

Introduction

The presence of a low molecular weight DNA polymerase in lower eukaryotes and plants is a controversial subject [for reviews see 4, 19]. Animal cell DNA polymerase β has a low molecular weight around 50 kDa and has been purified and characterized from different sources. It is inhibited by dideoxynucleoside triphosphates (ddNTPs), it is not affected by aphidicolin and it is remarkably resistant to the SH-reagent, N-ethylmaleimide, under conditions where the other plant or animal DNA polymerases are strongly inhibited [15]. Several lines of experimental evidence suggest a role for this enzyme in DNA repair [34]. The absence of a small polymerase in
wheat and *Vinca rosea*, as assayed in crude extracts by sucrose gradient centrifugation, led to the idea that this kind of enzyme was restricted to animal cells [7, 12]. An enzyme strictly similar to animal DNA polymerase β or present in all developmental stages has not been described in plant cells, although the presence of a small DNA polymerase has been reported in partially purified extracts from sugar beet and pea [9, 30, 33], lily [23] and cauliflower [35]. Other authors have classified a plant DNA polymerase as a β-like enzyme based on the strong affinity of this enzyme to plant chromatin [9, 33] which is the case of the nuclear animal enzyme.

Previously we have described the purification and characterization of multiple DNA polymerases (A, B, C) from quiescent wheat embryos [6]. These enzymes are most probably of nuclear origin since they differ from the mitochondrial [10] and chloroplastic [18] wheat DNA polymerases. Wheat DNA polymerase A, although it is not localized in the mitochondria, has some biochemical properties similar to those of animal DNA polymerase γ [18, 31]; DNA polymerase A seems also to be involved in the initiation of DNA synthesis [13]. Our first results [6] indicated that DNA polymerases B and C were α-like since both were inhibited by aphidicolin, but recent results allowed us to further characterize DNA polymerase B (M.C. Richard *et al.*, manuscript in preparation) and show that it resembles strongly the recently described DNA polymerase δ from animal and yeast [5]. DNA polymerase C was separated from the other wheat DNA polymerases since it eluted in a DEAE-cellulose column flow-through fraction. As the molecular weight of the DNA polymerase C varied in different preparations, we suspected a proteolytic degradation, a phenomenon already described in the case of spinach DNA polymerase α [22].

These results prompted us to modify the enzyme purification strategy and add several protease inhibitors during the purification procedure. In this article we show that, when wheat DNA polymerases were purified under these experimental conditions, two DNA polymerases were clearly resolved from the enzyme activity which was not retained in a DEAE-cellulose column, previously described as DNA polymerase C. Thus, a low and a high molecular weight DNA polymerase were purified to apparent homogeneity. A study of the biochemical and immunological characteristics of the high molecular weight (α-like) polymerase will be described elsewhere (manuscript in preparation). The low molecular weight DNA polymerase from wheat embryos (C1), described in this article, is a plant enzymatic form that shares many properties with animal DNA polymerase β, except that it is strongly inhibited by N-ethylmaleimide.

**Materials and methods**

**Materials**

Freshly prepared wheat germ was a kind gift from ‘Les Grands Moulins de Bordeaux’. Polynucleotides, oligonucleotides and calf thymus DNA were from Sigma Chem. Co., Boehringer and Pharmacia. Aphidicolin, heparin, dideoxythymidine (ddTTP) and N-ethylmaleimide were from Sigma. Phenylmethyl sulfonylfluoride (PMSF) was from Boehringer Mannheim. Leupeptin and pepstatin were from Sigma. Labeled precursors were from the Commissariat à l’Énergie Atomique-Saclay, from New England Nuclear and Amersham. DEAE-cellulose (DE-52) and phosphocellulose (P11) were from Whatman. HA-Ultragel was obtained from I.B.F. Biotechnics and Mono S (HR5) column was from Pharmacia. Gel electrophoresis reagents were purchased from Pharmacia. *Xenopus laevis* oocyte DNA polymerase α was purified as described before [37]. Rat liver DNA polymerase β was a kind gift of Dr. J.M. Rossignol (Villejuif-France).

**Methods**

**Purification of DNA polymerase C**

All purification steps were carried out at 4 °C. Buffer A contained 50 mM Tris-HCl pH 7.5, 1 mM 2-mercaptoethanol, 0.1 mM EDTA and