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CHANGES IN THE ACTIVITY OF POLY(ADENOSINE DIPHOSPHATE-RIBOSE) POLYMERASE DURING GERMINATION OF PEA

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Key Word Index—*Pisum sativum*; Leguminosae, pea; germination; nuclei; poly(ADPR) polymerase.

Abstract—Poly(adenosine diphosphate-ribose) polymerase activity has been detected in nuclei from pea seedlings. The reaction is a specific polymerization of the adenosine diphosphate-ribose moiety of NAD, giving an average chain length of 2.4 residues. The enzyme shows marked changes in activity during germination: it exhibits a clear peak between 24 and 36 hr of germination, after which the activity declines to the level observed in ungerminated seeds.

INTRODUCTION

It has been known for several years that eukaryotic organisms can bring about a polymerization of the adenosine diphosphate-ribose moiety of NAD to give poly(adenosine diphosphate-ribose) [1]. The reaction is mediated by poly(adenosine diphosphate-ribose) polymerase [poly(ADPR) polymerase]. The polymer is covalently bound to histones or non-histone proteins [1] although a proportion of the total polymer population may be free in the nucleoplasm [2]. The role of the covalent modification of proteins by poly(ADPR) is not at all clear, although currently, the weight of the available evidence suggests that it may have a role in DNA repair [3–6], possibly by regulating the activity of DNA ligase [7, 8].

Only relatively recently has there been any investigation of poly(ADPR) polymerase activity in plants. Its presence has been detected by cytological techniques in onion roots and seeds [9], and by conventional enzyme assay techniques in nuclei of wheat [10], rye [11] and tobacco [12]. However, there is some confusion concerning the latter paper, since the author stated that activity was measured by measuring incorporation of radioactivity from [^{14}C-*carbonyl*] NAD. (The carbonyl group is part of the nicotinamide moiety which is eliminated during the polymerization reaction.)

In this laboratory, we have been working on the germination of the garden pea (*Pisum sativum*). Under the conditions routinely used, there is a lag of some 30–35 hr before the onset of DNA replication in the embryo axis [13]. This system thus provides firstly a long period in which DNA replication does not occur, but during which it is likely that DNA repair can occur [14], and secondly, a relatively well-defined period in which DNA replication is initiated in part of the total cell population. We have therefore used this system in an investigation of poly(ADPR) polymerase activity.

RESULTS AND DISCUSSION

General features of the reaction

The optimum concentration of magnesium chloride was 12.5 mM and there was a sharp pH optimum at 7.9. Under these conditions, the enzyme exhibited its maximum rate at 12.5 μM NAD. The reaction was linear for 5–10 min and was almost completely inhibited by nicotinamide at 10 mM. These general features are similar to those seen in the enzyme from tobacco: pH 7.9; 10 mM Mg^{2+} [12] but differ slightly from those shown by the enzyme from wheat: 2 mM Mg^{2+} [10].

Specificity of the reaction

The radioactivity incorporated into TCA-insoluble material was not solubilized by deoxyribonuclease, ribonuclease or water, but was solubilized by sodium hydroxide and neutral hydroxylamine. If [^{14}C-*carbonyl*] NAD was used in the reaction mixture instead of [^{3}H-*adenine*] or [^{14}C-*adenine*] NAD, no incorporation of radioactivity into TCA-insoluble material occurred. These data thus indicate that the reaction was a specific polymerization of the adenosine-diphosphate-ribose moiety of NAD, with the elimination of nicotinamide, and that the product was not covalently linked to nucleic acids but probably was covalently linked to protein via a carboxyl ester linkage.

Chain length

Chain length of the oligomer synthesized by the enzyme was 2.42 ± 0.03 (mean of two determinations with range). Oligomers of very similar size are synthesized by the enzymes from wheat [10], rye [11] and mouse liver [2].

Changes in activity during germination

The changes in poly(ADPR) polymerase activity during germination are shown in Fig. 1. Low, but readily assayable activity was present in the embryo axis at least as early as 4 hr after the onset of imbibition. There was a very dramatic increase in activity between 16 and 35 hr after the onset of imbibition, following which activity fell to reach a value similar to that observed in the very early stages of germination. The pattern of change illustrated in Fig. 1 was completely reproducible between different

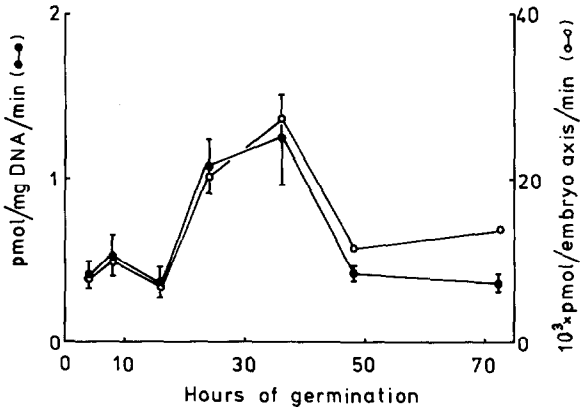

Fig. 1. Changes in poly(ADPR) polymerase activity in nuclei from pea embryo axes during germination. Assays were carried out under optimum conditions for 5 min (i.e. during the linear phase of the reaction).

batches of seeds, although the actual levels of activity varied between batches, giving rise to the relatively large error bars. The dramatic rise and fall of the enzyme's activity in pea contrasts with the situation in wheat, where the enzyme activity changes very little during germination [15]. The peak of activity in pea was approximately coincident with the initiation of DNA replication. However, this does not imply a role for the enzyme in DNA replication, for the following reasons.

Firstly, the pea embryo axis is not a synchronous population of cells, and so once DNA replication has been initiated in the axis, there are always some cells in the S-phase. An enzyme involved in DNA replication would not therefore be expected to show a marked decline in activity. Secondly, enzymes known to be involved in DNA replication, such as DNA polymerase-α, increase in activity during early germination of the pea and show no decrease after the onset of DNA replication [13]. This suggests that poly(ADPR) polymerase is involved in a process which is particularly active during germination, but not thereafter. It is known that the DNA of cereal seeds exhibits damage, and that this damage is repaired in the period immediately prior to the onset of DNA replication [14]. Similar data have been obtained in this laboratory for the DNA of carrot and pea seeds and in pea, the repair takes place between 24 and 32 hr after the onset of imbibition of the seed. The timing of the increase in poly(ADPR) polymerase activity in pea embryo axes is such that the enzyme could be involved in DNA repair.

EXPERIMENTAL

Growth of plants. Pea seeds (*Pisum sativum* L., cv 'Feltham First') were imbibed, germinated and grown at 20–22° as previously described [13]. At different times after the onset of imbibition, embryo axes were excised and used for extraction of nuclei.

Nuclei. These were prepared as described by Slater *et al.* [16]. Nuclei prepared in this way were contaminated by starch grains, but were free of all other cytoplasmic components, as judged by light microscopy and by PAGE of extracted nucleic acids [17].

Assays of poly(ADPR) polymerase. Nuclei were resuspended in 50 mM Tris–HCl, pH 7.9; 360 μl of suspension were assayed in a total vol. of 400 μl, containing 12.5 mM $MgCl_2$, 5 mM β-mercaptoethanol, 1 mM Na_2EDTA and, unless otherwise specified, 12.5 μM NAD ([^{3}H-*adenine*] NAD, 20 Ci/mmol or [^{14}C-*adenine*] NAD, 265 mCi/mmol). Assay mixtures were incubated at 25°; the reaction was stopped by the addition of an equal vol. of ice-cold 10% (w/v) TCA. One hr later, the ppts were collected on Whatman GF-C glass-fibre filters. The filters were washed (×2) with 5% (w/v) TCA and once with EtOH. The filters were then dried, and assayed for radioactivity in a toluene-PPO-POPOP scintillation cocktail.

Specificity of incorporation was tested firstly by hydrolysis of the labelled products with NaOH, NH_2OH, deoxyribonuclease and RNase as described by Willmitzer [12] and secondly by using NAD labelled in the carbonyl group of the nicotinamide moiety instead of in the adenosine moiety. The chain length of the newly synthesised oligomers was measured as described by Whitby *et al.* [10].

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