Phytochemistry, 1979, Vol. 18, pp. 1873–1874. Pergamon Press Ltd. Printed in England.

SHORT REPORTS

PARTIAL PURIFICATION AND PROPERTIES OF NUCLEASE I FROM BARLEY MALT

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(*Received* 6 *January* 1979)

Key Word Index—*Hordeum vulgare*; Gramineae; barley; malt; nuclease; 3′-nucleotidase.

Abstract—A sugar-unspecific nuclease has been purified 260-fold from barley malt diastase. The enzyme, a glycoprotein of 37000 MW, is highly active on single-stranded polynucleotides at pH 5–6. The nuclease is inhibited by several adenine nucleotides, and it binds weakly to NADP-agarose and ATP-agarose.

INTRODUCTION

Plant nuclease I (nucleate endonuclease, EC 3.1.4.9) is an enzyme that catalyses the hydrolysis of both DNA and RNA and also exhibits 3′-nucleotidase activity [1]. Partially purified preparations have been obtained from several plants, but only the mung bean nuclease [2] has been obtained in a homogeneous form. Nuclease I has not been purified previously from germinated barley, although it has been known for 25 years that extracts of this material contain 3′-nucleotidase activity [3] and a Mg^{2+}-independent, acid hydrolase activity for low MW DNA [4]. In this communication we describe a partial purification of nuclease I from barley malt diastase, a readily available crude source of this enzyme, and a study of its interaction with soluble and immobilized nucleotides.

RESULTS AND DISCUSSION

The purification of the sugar-unspecific nuclease from barley malt is summarized in Table 1. The specific activity of the final preparation was 260-fold greater than that of the crude extract, and the yield was 24%. The following relative rates of hydrolysis of various substrates were observed with the purified enzyme: partially degraded DNA, 100; single-stranded (ss) DNA, 55; RNA, 27; and 3′-AMP, 77. The purified enzyme preparation is still contaminated with a small amount of acid phosphatase activity. β-Glycerophosphate was hydrolysed at 2% of the rate of partially degraded DNA. The pH values for optimal activity of the barley malt enzyme with ss DNA, RNA, and 3′-AMP were found to be 5, 5.8 and 7.8, respectively. Preparations of nuclease I from most plants are active on polynucleotides in slightly acidic solution and hydrolyse 3′-nucleotides over a wide range of pH, with maximal activity near neutrality.

Mung bean nuclease I is a glycoprotein containing 29% carbohydrate [2], and carbohydrate is also present in the partially purified preparation of nuclease I from barley malt. Fraction IV contained 37 μg carbohydrate/mg protein. The enzyme was found to bind quantitatively to concanavalin A-Sepharose and it could be completely eluted with 0.3 M α-methyl-D-mannoside. The specific interaction with the immobilized lectin indicates that barley nuclease I is also a glycoprotein. The MW of the enzyme was estimated to be 37000 by gel filtration. This is in the range of 31000 to 44000 previously reported for nuclease I from various plants [1, 5, 6].

Table 1. Summary of the partial purification of nuclease I from barley malt

Fraction	DNase activity (units)	Specific activity (units/ml·A_{280})
I Crude extract	211	0.0081
II Ammonium sulfate	148	0.045
III SP-Sephadex	83	0.82
IV Sephadex G-75	50	2.15

A previous report [7] from this laboratory described the inhibition of a similar nuclease from tobacco cell cultures [8] by various nucleotides. The barley nuclease is also inhibited by nucleotides, although the degree of inhibition is not as great. ADP-ribose, ADP-glucose, NAD, and 2′-AMP produced <25% inhibition of the DNase activity when tested at 0.5 mM. More inhibitory nucleotides included 5′-AMP, 32% inhibition; NADP, 40%; 2′,5′-ADP, 69%; 2′-P-ADP-ribose, 74%; ADP, 94%; and ATP, 98%. ATP is also a potent inhibitor of nuclease I from tobacco cell cultures and mung bean sprouts [7].

NADP-agarose is an effective affinity adsorbent for nuclease I from tobacco cultures and several other nucleases active on RNA [9]. However, under varying conditions of adsorption, from 25 to 65% of an applied sample of barley malt nuclease failed to bind to NADP-agarose. The bound enzyme was eluted at pH 5.5 by 5 mM 3′-AMP, but not by 5 mM NaCl. This weak biospecific interaction is similar to that observed with mung bean nuclease [9], and it may reflect the fact that NADP

inhibits the nuclease from barley less than that from tobacco cultures. The nicotinamide-free analog of NADP, 2'-P-ADP-ribose, inhibits barley nuclease I to about the same extent that NADP inhibits the tobacco nuclease, and it is possible that the former nucleotide could be immobilized to yield an effective affinity adsorbent for nuclease I from barley and other plant species. Interaction of the barley malt nuclease with ribose-linked ATP-agarose [10] was also examined. Almost half of the applied nuclease sample failed to bind to ATP immobilized by this method. In contrast, Sitz *et al.* [11] have recently shown that S_1 nuclease, a related fungal nuclease, is readily adsorbed by adenine-linked ATP-Sepharose. These observations suggest that an intact ribose moiety may be required in nucleotide ligands that bind strongly to the barley nuclease.

EXPERIMENTAL

Enzyme assays. Nucleases active on DNA and RNA were assayed as described previously [7], except that a pH of 5 was used in the reaction mixture. Hydrolytic activities on 3'-AMP and β-glycerophosphate were determined as described in ref. [8]. One unit (U) of enzyme activity is defined as the amount that catalyses the formation of 1 μmol of product per min under the standard conditions. Tris, acetate and MES buffers of pH 4–9 were used to determine the pH optima of the purified enzyme with various substrates.

Enzyme purification. All steps were carried out at 0–4°, and all centrifugations were performed at 8000 *g*, 30 min. Fractions were assayed for nuclease activity by the standard method with ss DNA as substrate. Barley malt diastase (Sigma, A-6755), 120 g, was stirred in 400 ml 0.1 M Tris–acetate buffer (pH 7.6) for 40 min. The suspension was centrifuged and the supernatant soln (fraction I) was subjected to $(NH_4)_2SO_4$ fractionation. The material precipitating between 45 and 80% satn was dissolved in 0.05 M Na acetate buffer (pH 4.5) containing 1 mM $ZnCl_2$ and 2 mM 2-mercaptoethanol (buffer A, pH 4.5). This soln (fraction II) was dialysed against buffer A, pH 4.5, clarified by centrifugation, and applied to a sulfopropyl-Sephadex column (1 × 10.5 cm) equilibrated with buffer A, pH 4.5. The column was washed with 32 ml buffer A, pH 4.5 and 32 ml buffer A, pH 5.5. The nuclease was eluted with 16 ml buffer A, pH 6 containing 0.1 M NaCl and then dialysed against buffer A, pH 5.5 containing 15% (v/v) glycerol. This soln (fraction III) was applied to a Sephadex G-75 column (3 × 94 cm) equilibrated with buffer A, pH 5.5. Fractions, 7 ml, were collected at a flow rate of 40 ml/hr. Tubes with high DNase activity were pooled (fraction IV), and this material was used in the characterization studies.

Inhibition by nucleotides. The standard assay method was used. Reaction mixtures containing ss DNA substrate and 20 μU enzyme were supplemented with nucleotides at 0.5 mM.

Interaction with immobilized nucleotides was measured in Pasteur pipets that contained 0.5 ml of resin equilibrated with 0.01 M Na acetate buffer (pH 5.5) containing 0.1 mM $ZnCl_2$ and 2 mM 2-mercaptoethanol (buffer B). The nuclease, 0.2–1 U in 1 ml buffer B, was applied to the column, which was then washed to remove unadsorbed enzyme. The nuclease was desorbed with dilute buffer solns (pH 5.5 or 7.6) containing increasing concns of substrate (3'-AMP) or NaCl. Binding to concanavalin A-Sepharose (Pharmacia) was tested in 0.01 M Tris acetate buffer (pH 7.6) containing 5 mM $MnCl_2$ and 5 mM $CaCl_2$, and the enzyme was desorbed with the same buffer containing α-methyl-D-mannoside (0.05–0.3 M). Fractions eluted from the columns were assayed for nuclease activity on ss DNA. Recovery of enzyme activity from affinity columns ranged from 80 to 100%.

Other methods. The method of ref. [12] was used for Pi analysis, A_{280} was used as a measure of protein concn and carbohydrate was determined by the method of ref. [13] using glucose as a reference. The MW of the nuclease was estimated by gel filtration [14] on Sephadex G-75, with BSA, ovalbumin, carbonic anhydrase and cytochrome *c* as standards. The methods of refs. [9], [10] and [15] were used for the prepn of NADP-agarose, ATP-agarose and partially degraded ss DNA, respectively.

Acknowledgement—This paper is Journal Article No. 982 of the North Dakota Agricultural Experiment Station.

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