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Nucleases in Chloroplast of Barley

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Abstract. Two barley chloroplast nuclease fractions were separated by the affinity chromatography and gel electrophoresis. Both were about 2 times more active to RNA than to native DNA and about half as active to denaturated DNA as to native DNA. Both fractions were as active to UV-irradiated (270 J m^{-2}) native DNA as to intact DNA but their action was inhibited by apurinic sites. The enzyme activities were inhibited by high concentrations of EDTA, NaCl, Mn^{2+}, Ca^{2+}, Zn^{2+} ions and by N-ethylmaleimide. They do not require Mg^{2+} ions but are stimulated or at higher concentration inhibited by their presence. Both RNase and DNase were active over a wide pH range (5.5–9), the optimum for DNase action in the presence of Mg^{2+} being 6.5, for RNA decomposing activity at pH 8.0. As no mononucleotides were detected in acid soluble form, it seems likely that DNase acts in the endonucleolytic way.

Several nucleases that degraded native and denaturated DNA have been described in higher plants. It is believed that they are involved in DNA replication and in the process of aging (WYEN *et al.* 1971, JENNS and BRYANT 1978). Except for one, purified from malt diastase (LIAO 1977) all of them are sugar nonspecific, *i.e.* they also attack RNA and both activities were inseparable in spite of a high degree of purification. Besides the RNase and DNase activities, some of them — *e.g.* the best characterized Nuclease I, possesses also the phosphomonoesterase as well as 3′ nucleotidase activities. Numerous differences have been detected among nucleases of different plant species or even of one species with respect to specifities towards various substrates, pH optimum, requirements of bivalent cations, inhibition by EDTA *etc.* (*cf.* WILSON 1975). Several data are also known about the subcellular localization of plant nucleases. In nuclei of rye, an endonuclease hydrolyzing both DNA and RNA with the pH optimum of 5.4—7.4 has been recently isolated (BORUCKA-MANKIEWICZ and SZARKOWSKI 1977). Another endonuclease, sensitive to actinomycin, supposed to form primers for DNA polymerase, was detected in nuclei of *Tradescantia* pollen (KLIGMAN and TAKATS 1975). In barley, one nuclease hydrolyzing both RNA and DNA was localized in chromatin (SRIVASTAVA *et al.* 1971) whereas another one, differing in the pH optimum, was detected in the soluble fraction of cells (SRIVASTAVA 1968). Yet, a soluble type of DNA degrading nuclease was isolated from barley malt diastase (BRAWERMAN and CHARGAFF 1954), differing from the chromatin nuclease in inactivity towards RNA. Besides, it pos-

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sessed phosphomonoesterase activity, required Mg^{2+} and Mn^{2+} ions, was stabilized by Ca^{2+} and was, therefore, comparable to pancreatic DNase I (LIAO 1977).

The nucleases described in this paper differ in some respects from barley nucleases known so far. Their partial purification and some characteristics are described.

MATERIAL AND METHODS

Chloroplasts were isolated from 500—1500 g of 10—14 days-old barley leaves (*Hordeum vulgare* cv. Ametyst). The procedure, that essentially followed the method described by TRAVIS (1977), included homogenization in Honda buffer (2.5% Ficoll, 0.25 M sucrose, 5% dextran T 40, 1 mM $MgCl_2$, 4 mM 2-mercaptoethanol, 0.025 M Tris-HCl buffer pH 7.8), low speed centrifugation for removing cell debris, and centrifugation in the discontinuous sucrose gradients (20%, 45% and 60% sucrose in Honda buffer) at 24 000 rpm in SW 27 Beckman rotor for 2 h at 4 °C. Purity of chloroplast suspension was checked in an optical microscope after staining the residual nuclei with methylene green.

Chloroplasts free of nuclei were suspended in 0.01 M Tris-HCl buffer pH 7.8 containing 0.001 M $MgCl_2$ and kept in a refrigerator. They were destroyed by dialysis for 30 min against distilled water, additionally dialyzed against buffer A (0.05 M Tris-HCl buffer pH 8 with 0.1 mM 2-mercaptoethanol and 10% glycerol). Residual intact chloroplasts and chloroplast debris were removed by centrifugation at 16 000 × *g* and the supernatant was used as the "crude chloroplast extract" for further purification of enzymes.

DNase Assay with [^{3}H]DNA

5-[^{3}H] thymidine-DNA was isolated from *E. coli* B/3 thy$^-$ with specific activity of about 10^3 counts . min^{-1} μg^{-1}. Modified DNAs were prepared by irradiation of native [^{3}H] DNA with 270 J m^{-2} UV light or by alkylation with 50 mM methyl methanesulphonate (end conc.) for 30 min at 37 °C fol lowed by depurination at 50 °C for 4 h.

Denaturation of DNA was carried out by heating DNA samples for 10 min at 100 °C and rapid cooling in an icebath. The incubation mixture contained 100 µl [^{3}H] DNA solution (10 µg ml^{-1}) with 0.1 M NaCl, 3 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 0.02 mg ml^{-1} yeast tRNA, 0.5 µg ml^{-1} human serum albumine in 50 mM HEPES buffer pH 8, and up to 50 µl of enzyme-containing material (or buffer A in controls). The mixture was incubated for 50 min at 37 °C.

The solution was cooled and DNA precipitated by adding 100 µl of 0.8 M perchloric acid. Radioactivity of acid soluble fraction was measured as described earlier (ŠVACHULOVÁ *et al.* 1978). Enzyme activity was expressed as the percentage of acid soluble radioactivity released from the total radioactivity of the substrate.

RNase and DNase Assay with Unlabelled Substrates

was carried out essentially according to SRIVASTAVA *et al.* (1971): 100—150 µl of enzyme-containing solution was incubated for 1—6 h at 37 °C with 100 to 200 µl of yeast RNA (2 mg ml^{-1}) or calf thymus DNA (2 mg ml^{-1}) dissolved in 0.05 M Tris-HCl buffer pH 7, with or without 9 mM $MgCl_2$. The

incubation was stopped by adding 2.5 volumes of $HClO_4$ + ethanol (1 : 13 v/v), cooled to 20 °C for 2—3 h and centrifuged at 9 000 × *g* for 30 min at 4 °C. The optical density of the supernatant at 260 nm was read against control prepared in the same way but with the buffer instead of the enzyme solution. Enzyme activity was expressed as the percentage of the total absorbance at 260 nm released into the acid/alcohol soluble fraction.

Purification of Enzymes by Affinity Chromatography

was carried out according to HADI *et al.* (1973) on a column (1 × 6 cm) of DNA-cellulose. About 50% binding of calf thymus DNA to cellulose (Schlecher + Schüll) was obtained by the method of ALBERTS *et al.* (1968). About 45 ml of the "crude chloroplast extract" was applied onto the column, the column was washed with 6 ml of buffer Ad an eluted gradually with 0.1 M, 0.25 M and 0.5 M NaCl dissolved in buffer A (6 ml each). Fractions collected during the application of the sample (4 × 11 ml), washing of and elution from the column were concentrated about 4 times of Diaflo UM-10 ultrafilters under pressure of N_2.

Acrylamide Gel Electrophoresis

of the concentrated fractions was made according to the method described by DAVIS (1964) and modified by NOVACKÝ *et al.* (1966).

Isoelectric Focusing Gel Electrophoresis

was carried out according to the method described by RADMAN (1976). Fractions after the acrylamide gel electrophoresis having nucleolytic activity towards DNA were focussed in a pH gradient formed in gel containing 5% acrylamide, 0.2% methylenebisacrylamide, 4 M urea and 2% ampholine pH 3.5—10 (LKB, Uppsala, Sweden). Polymerization was initiated by the addition of 3 ml of 5% Temed and 3 ml of ammonium persulfate per ml of the gel solution. The top reservoir containing 0.2% H_2SO_4 was connected with the anode, the bottom reservoir containing 2% ethylenediamine with the catode.

Molecular Weight

of the purified enzyme was determined on a Sephadex G-75 column (1 cm × 34 cm) using cytochrome c (m.w. 12 400), ovalbumine (m.w.45 000) and bovine serum albumine (m.w. 67 000) as standards (*cf.* ŠVACHULOVÁ *et al.* 1978).

The Mode of Nuclease Action

was estimated by the analysis of DNA degradation products on a Sephadex G-10 column (1.3 × 82 cm). Calf thymus DNA (0.5 ml, 2.2 mg ml^{-1}) was incubated with 0.3 ml of the enzyme solution at 37 °C for 3 or 6 h and the whole sample was applied into the column and eluted with SSC (0.15 M NaCl, Na citrate — pH 7.0) at a flow rate of 7.5 ml h^{-1} and at room temperature. The column was calibrated roughly by passing through the undegraded calf thymus DNA (1.1 mg), 1 mg 2′-deoxythymidine and guanylic acid (1 mg).

Proteins were determined spectrophotometrically according to the method described by BRADFORD (1976).

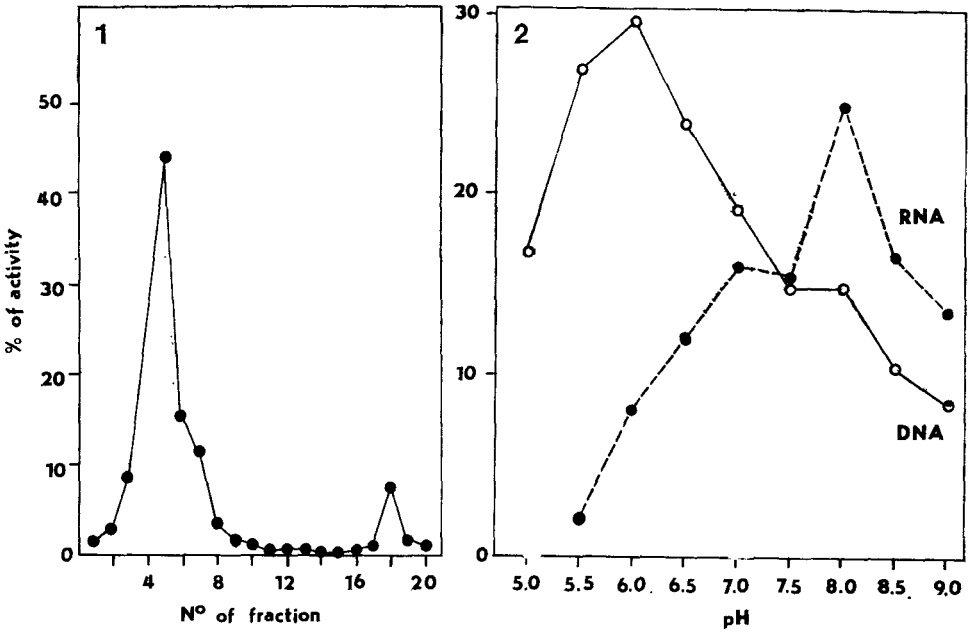

Fig. 1. Separation by polyacrylamide gel electrophoresis of nucleases active towards native [^{3}H]DNA. Samples (200 μl, about 20 μg per gel) of chloroplast extract were previously purified on DNA-cellulose column eluted with 0.1 M NaCl. Gels were sliced to 20 pieces and each slice was eluted with 200 μl of buffer A. 40 μl of eluates were used per one assay.

Fig. 2. Dependence of enzyme activity, purified as peak I by polyacrylamide gel electrophoresis (*cf.* Fig. 1.), on pH of the incubation mixture. Assay for DNase activity was carried out with *E. coli* [^{3}H] DNA (100 μl per assay) and pooled eluates of peak I (40 μl per assay). Activity towards RNA was measured spectrophotometrically with yeast RNA (125 μl, 2 mg ml^{-1}) incubated with pooled eluates of peak I (50—100 μl) for 1—3 h.

RESULTS

Enzyme Separation

The "crude chloroplast extract" obtained after the chloroplast destruction and removal of the debris by centrifugation was applied onto DNA-cellulose. Fractions obtained after the application of the sample, washing the column and elution with 0.1 M, 0.25 M and 0.5 M NaCl were collected, concentrated on Diaflo ultrafilters and applied onto acrylamide gels so that all tubes of one electrophoretic device contained samples of the same fractions. After the electrophoresis, each gel was cut into 20 slices, those of the same order were pooled, disintegrated mechanically, eluted with buffer A (200 μl per slice) for 48 h at 4 °C and the eluates were tested for the nuclease activity towards native [^{3}H] DNA. Main peak (peak I — *cf.* Fig. 1) of nuclease activity was repeatedly found at slices No 5—9 analysed fractions obtained after the affinity chromatography. Most of these "fractions" contained also another less active nuclease eluted from slices No. 16—18 of polyacrylamide gels (peak II in Fig. 1). Only one band of nuclease activity of peak I was found in isoelectric focussing gels (pI ~ 9.3). Low recovery of activity (about 3%), however, caused probably by the presence of unpolymerized acrylamide, did not permit us to exclude the presence of another protein of lower nuclease activity in this peak.

TABLE 1

Purification of nucleases from barley chloroplasts. The activity was measured with native *E. coli* [^{3}H] DNA. One unit represents an amount of enzyme containing solution (in μl) which releases 10% of acid soluble [^{3}H] material after the incubation with [^{3}H] DNA substrate

Purification step	Protein content [μg ml^{-1}]	Specific activity [units μg^{-1} proteins]	Purification factor
1. Crude chloroplast extract	23.8	2.4	1
2. DNA-cellulose chromatography			
Sample application:			
Fraction 1	4.0	12.1	5.1
Fraction 2	16.5	7.6	3.1
Fraction 3	16.5	7.7	3.2
Fraction 4	18.5	7.9	3.2
Washing with buffer A	15.0	13.7	5.7
Elution with 0.1 M NaCl	4.0	22.5	9.7
Elution with 0.25 M NaCl	4.5	18.4	7.7
Elution with 0.5 M NaCl	3.0	8.6	3.5
3. Gel electrophoresis, peak I	1.0	400.0	167.0

As shown in Table 1 the nuclease of the peak I was purified about 167 times as compared to the specific activity detected in the "crude chloroplast extract".

Properties of Chloroplast Nucleases

Eluates of both nuclease peaks in acrylamide gels were tested towards various substrates (Table 2). Both peaks were about 2 times more active to RNA than native calf thymus DNA and about twice as active to native

TABLE 2

Substrate specificity of chloroplast nucleases purified by the affinity chromatography and by acrylamide gel electrophoresis. Data represent the activities obtained after testing the peaks I and II separated in polyacrylamide gels (Fig. 1)

Substrate	% of substrate released into the soluble fraction	
	Peak I	Peak II
E. coli [^{3}H] DNA — native	18.9	7.4
E. coli [^{3}H] DNA — heat denaturated	8.6	not determined
E. coli UV-irradiated [^{3}H] DNA — native	15.4	5.7
E. coli depurinated [^{3}H] DNA — native	2.0	1.4
Calf thymus DNA — native	13.5	3.0
Yeast RNA	34.4	17.6

Data represent means of several independent measurements. *E. coli* [^{3}H] DNA was mostly incubated for 45 min at 37 °C at the presence of 9 mM $MgCl_2$ and 50 μl of enzyme per 100 μl of the substrate. Calf thymus DNA and yeast RNA (2 mg ml^{-1}) was incubated with 250 μl of enzyme at presence of 9 mM $MgCl_2$ for 3 h at 37 °C.

TABLE 3

Dependence of chloroplast nuclease activity on the concentration of Mg^{2+}, NaCl and EDTA in the incubation mixture. EDTA and NaCl effects were tested in the presence of 9 mM $MgCl_2$. DNase activity was determined with the *E. coli* [^{3}H] DNA, RNase activity was detected spectrophotometrically after the incubation period of 3 h

Agent	Conc. 10^3 M	Peak I				Peak II			
		DNase [%]		RNase [%]		DNase [%]		RNase [%]	
		pH 8	pH 6.5	pH 8	pH 6.5	pH 8	pH 6.5	pH 8	pH 6.5
$MgCl_2$	0	1.8	8.2	16.6	12.0	2.8	3.8	3.4	9.4
	3	6.0	8.9	10.3	8.9	4.8	4.8	7.7	5.2
	9	6.4	11.6	11.1	7.7	20.8	3.8	7.4	4.1
	18	—	—	8.3	—	12.6	4.3	—	3.4
EDTA	0	10.0		11.0		20.8	4.0		4.1
	20	2.5		2.9		1.8	0.2		0.1
	40	2.1				0.2	0.2		0.1
NaCl	50	10.6				15.6			
	100	9.9				20.0			
	200	4.2				3.8			
	300	2.2				1.8			

than to heat denaturated *E. coli* DNA. Lesions induced in the substrate *E. coli* DNA by the UV-light did not influence the activity of both nucleases but apurinic sites evidently inhibited their action.

Dependence of the nuclease activity on pH of the incubation mixture was determined only for nuclease eluted as the peak I of the gel. The incubation mixture was adjusted to the appropriate pH by means of 0.01 or 0.001 M NaOH and HCl before incubation and checked after the incubation. Both the DNase and the activity towards RNA were active over a wide range of pH (5—9), however their pH-optima differed significantly being about 8 for the RNA and about 6 for the DNA substrates. The second peak of nuclease activity (peak II) in polyacrylamide gel revealed a similar range of activity as the nuclease of the peak I; however, the pH optima were not detected.

Both activities towards DNA and RNA of the elution peak I were inhibited by 5 mM $CaCl_2$, 10 mM $ZnCl_2$ and 10 mM $MnCl_2$. Ethylmaleimide, the inhibitor of SH dependent enzymes decreased the DNase activity towards [^{3}H] DNA about 2—3 times at the concentrations of 25 mM and 50 mM. Activities of elution peaks towards both DNA and RNA were inhibited strongly by 20 mM to 40 mM EDTA (Table 3). DNase activity of both elution peaks I and II was inhibited by sodium chloride at concentrations higher than 100 mM (Table 3). Activity towards RNA was not tested in this respect. DNase activities of both peaks were also stimulated by Mg^{2+} ions especially at pH 8, 9 mM being an optimal concentration. Activities towards RNA were slightly more active in the absence of Mg^{2+} (Table 3).

Nucleases of both peaks separated in acrylamide gels were rather stable when bound to a chloroplast structure in isolated chloroplasts or their fragments. They could be kept for several months in the refrigerator without

any substantial loss of activity. They maintained their activities also in fractions collected after affinity chromatography, probably due to the presence of DNA or its fragments eluted simultaneously from the column. The DNase activity of the peak I from the acrylamide gel was fully destroyed on heating at 50 °C for 5—10 min before testing, whereas the activity towards RNA was stable at this temperature.

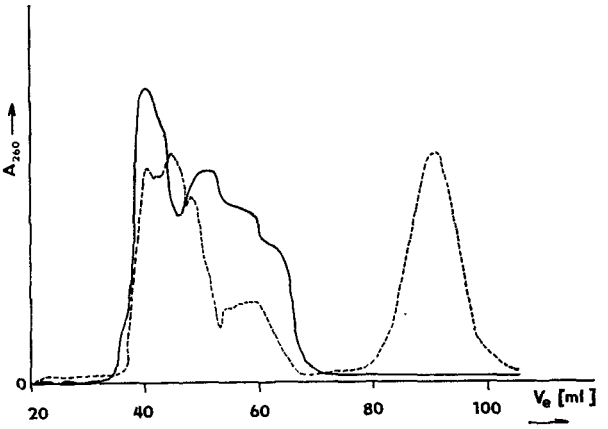

Fig. 3. Chromatography of DNA degradation products yielded after the incubation of DNA with chloroplast nuclease purified on DNA cellulose and by polyacrylamide gel electrophoresis. Chromatography was carried out with Sephadex G-10 column (1.3 × 85 cm) with a flow rate 10.5 ml h^{-1}. Full line represents a sample of calf thymus DNA (0.5 ml, 2.2 mg ml^{-1}) incubated with 0.3 ml nuclease solution (peak I — *cf.* Fig. 1) for 3 h at 37 °C. Dotted line represents control calf thymus DNA (0.5 ml, 2.2 mg ml^{-1}) mixed with 0.3 ml solution containing 1 mg guanosine-3′-monophosphoric acid and 1 mg thymidine in SSC.

Molecular weight of the nuclease in the peak I of polyacrylamide gel, as determined by Sephadex G-75 gel filtration was about 35 000.

It is evident from the chromatography profiles in Sephadex G-10 (Fig. 3) that native calf thymus DNA was hydrolyzed to oligonucleotides after incubation with the nuclease eluted as the peak I of the gel. Virtually no mononucleotides were detected even after 6 h of incubation. It may therefore be assumed that this chloroplast nuclease hydrolyzes DNA in an endonucleolytic manner.

DISCUSSION

Two electrophoretically separable nucleases were detected in barley chloroplasts, which degraded both RNA and native or denaturated DNA, were inhibited by EDTA, and by N-ethylmaleimide, by high concentrations of NaCl, Ca^{2+}, Mn^{2+} and Zn^{2+} ions. They were not inhibited by the DNA lesions induced by UV light, however, their activity was decreased in the presence of apurinic sites in substrate DNA. Similarities in the behaviour of both nucleases may indicate that they represent only two activities of the same enzyme, the molecular weight of which is similar to other plant sugar nonspecific nucleases. On the other hand, the different response of activities of both peaks towards DNA and RNA to the concentrations of $MgCl_2$ as well as their different pH optima indicate that the enzymes were only par-

tially separated and in fact both peaks (or at least peak I) represent a mixture of RNA and DNA degrading enzymes nonseparated from each other by our precedure. Althought it is commonly believed that sugar nonspecificity is a general feature of plant nucleases (*cf.* WILSON 1975), LIAO (1977) isolated DNase from malt diastase, inactive to RNA. Finally in another work we have separated (by a similar technique as described) chloroplast nuclease predominantly active to UV-irradiated DNA, which was inactive to RNA (VELEMÍNSKÝ *et al.* 1979).

As it has been already mentioned, several nucleases were described in barley. The chloroplast nuclease(s) seems to differ from them as far as the published data enable a comparison. Although the pH optimum of malt DNase (LIAO 1977) and our chloroplast nuclease(s) differed only slightly, the activity of the latter was only poorly influenced by Mg^{2+} and was inhibited by Mn^{2+}. Moreover, chloroplast nuclease was sugar nonspecific and was not stabilized but inhibited by Ca^{2+}.

The chromatin nuclease (SRIVASTAVA *et al.* 1971) differed from the chloroplast nuclease in being about two times more active to the denatured DNA than to native DNA and in having similar pH optima for RNA and DNA, different from that found for the chloroplast enzyme. Moreover, the chromatin nuclease was not inhibited by EDTA and N-ethylmaleimide.

The comparison of chloroplast nuclease(s) with soluble nuclease (SRIVASTAVA 1968) and with the "DNase" of BRAWERMAN and CHARGAFF (1954) is difficult due to the only few common characteristics determined. Moreover, their pH optima are so close that they might be supposed to be one enzyme. However, they differ in stability. The "DNase" (BRAWERMAN and CHARGAFF 1954) seems to be less stable in the solution even at low temperature, the "soluble nuclease" is more thermostable (SRIVASTAVA 1968). Furthermore, the DNase requires the presence of Mg^{2+} ions (BRAWERMAN and CHARGAFF 1954).

No enzyme similar in all respects to chloroplast nuclease is known even in other mono- or dicotyledoneous plants. Rye nuclear nuclease, although it has similar higher affinity to native than to denatured DNA, differs in the sensitivity to metals, to EDTA and higher temperature (BORUSKA-MANKIEWICZ and SZARKOWSKI 1977). Most plant nucleases including the best characterized Nuclease I of mung bean (SUNG and LASKOWSKI 1962, WALTERS and LORING 1966), avena (WYEN *et al.* 1971), corn (WILSON 1968) or wheat (HANSON and FAIRLEY 1969), were more active to the denatured DNA. Similarly to most of these nucleases, chloroplast nuclease (peak I) has a molecular weight around 35 000 and seems to produce predominantly oligonucleotides.

REFERENCES

ALBERTS, B. M., AMODIO, F. J., JENKINS, M., GUTMANN, E. D., FERRIS, F. L.: Studies with DNA-cellulose chromatography. I. DNA-binding proteins from *Escherichia coli*. — Cold Spring Harbor Symp. Quant. Biol. **33** : 289—292, 1968.

BORUCKA-MANKIEWICZ, M., SZARKOWSKI, J. W.: Purification and some properties of a nuclease from rye germ nuclei. — Acta biochim. polonica **24** : 289—299, 1977.

BRADFORD, M. M.: A rapid and sensitive method for the quantition of microgram quantities of protein utilizing the principle of protein-dye binding. — Anal. Biochem. **72** : 248—254, 1976.

BRAWERMAN, G., CHARGAFF, E.: On a deoxyribonuclease from germinating barley. — J. biol. Chem. **210** : 445—454, 1954.

DAVIS, B. J.: Disc electrophoresis II. Method and application to human serum proteins. — Ann. N. Y. Acad. Sci. **121** : 407—427, 1964.

HADI, S. M., KIRTIKAR, D., GOLDTHWAIT, D. A.: Endonuclease II of *Escherichia coli*. Degradation of double and single-stranded deoxyribonucleic acid. — Biochemistry **12** : 2747—2754, 1973.

HANSON, P. M., FAIRSLEY, J. L.: Enzymes of nucleic acid metabolism from wheat seedlings I. Purification and general properties of associated deoxyribonuclease, ribonuclease and 3′ nucleotidase activities. — J. biol. Chem. **244** : 2440—2449, 1969.

JENNS, S. M., BRYANT, J. H.: Correlation between deoxyribonuclease activity and DNA replication in the embryonic axes of germinating peas. — Planta **138** : 99—103, 1978.

KLIGMAN, I. H. L., TAKATS, S. T.: An actinomycin-sensitive endonuclease associated with DNA synthesis in *Tradescantia* nuclei. — Exp. Cell Res. **95** : 176—190, 1975.

LIAO, T. H.: Isolation and characterization of multiple forms of malt deoxyribonuclease. — Phytochemistry **16** : 1469—1474, 1977.

NOVACKÝ, K., MACKO, V., HÄSSLER, J.: [Notes to the disc electrophoresis of plant proteins on acrylamide gel.] In Slovak. — Biológia **21** : 64—68, 1966.

RADMAN, M.: An endonuclease from *Escherichia coli* that introduces single polynucleotide chain scissions in ultraviolet-irradiated DNA. — J. biol. Chem. **251** : 1438—1445, 1976.

SRIVASTAVA, B. I. S.: Studies on chromatin of barley leaves during senescence. — Biochem. J. **110** : 683—686, 1968.

SRIVASTAVA, B. I. S., MATSUMOTO, H., CHADHA, K. C.: Studies on chromatin-dissociated nuclease from barley leaves. — Plant Cell Physiol. **12** : 609—618, 1971.

SUNG, S. C., LASKOWSKI, M., SR.: A nuclease from mung bean sprouts. — J. biol. Chem. **237** : 506 to 511, 1962.

ŠVACHULOVÁ, J., ŠATAVA, J., VELEMÍNSKÝ, J.: A barley endonuclease specific for apurinic DNA. Isolation and partial characterization. — Eur. J. Biochem. **87** : 215—220, 1978.

TRAVIS, D. M.: Molecular properties of chloroplast and nuclear DNA from *Mimulus cardinalis*. — Biochem. Physiol. Pflanzen **171** : 109—126, 1977.

VELEMÍNSKÝ, J., ŠVACHULOVÁ, J., ŠATAVA, J.: Endonucleases for UV-irradiated and depurinated DNA in barley chloroplasts. — Nucl. Acid Res. **8**: in press, 1980.

WALTERS, T. L., LORING, H. S.: Enzymes of nucleic acid metabolism from mung bean sprouts. I. Fractionation and concentration of phosphomonoesterase, ribonuclease M_1 and M_2, 3′ nucleotidase and deoxyribonuclease. — J. biol. Chem. **241** : 2870—2875, 1966.

WILSON, C. M.: Plant nucleases. — Annu. Rev. Plant Physiol. **26** : 187—208, 1975.

WILSON, C. M.: Plant nucleases I. Separation and purification of two ribonucleases and one nuclease from corn. — Plant Physiol. **43** : 1332—1338, 1968.

WYEN, N. V., ERDEI, S., FARKAS, G. L.: Isolation from *Avena* leaf tissues of a nuclease with the same type of specificity towards RNA and DNA. — Biochim. biophys. Acta **232** : 472—483, 1971.