Short Communication

Plant Nucleases

V. SURVEY OF CORN RIBONUCLEASE II ISOENZYMES

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ABSTRACT

Corn (*Zea mays* L.) ribonuclease II of the root microsomal fraction was isolated from 11 inbreds and seven hybrids. Polyacrylamide gel electrophoresis revealed a total of five bands of activity among corn lines tested. Most inbreds had only one isoenzyme, but three had two isoenzymes. The hybrids tested contained all of the isoenzymes found in the parental inbreds.

Corn roots contain at least three distinct types of nucleases that degrade RNA (14, 16). Corn RNase II (EC 3.1.4.23 [1972]) is distinguished from the soluble corn RNase I (also EC 3.1.4.23) by a higher pH optimum and its location on a microsomal fraction. Nuclease I (EC 3.1.4.9) acts on both RNA and DNA and is unrelated to the RNases. I previously reported that the inbreds WF9 and M14 had different RNase II isoenzymes, whereas the hybrid WF9 × M14 contained both isoenzymes (14). No isoenzymes of corn RNase I have yet been detected among a number of inbreds (14). The nature of the multiple molecular forms of corn nuclease I (14) has not been determined (G. Apel, unpublished). I wish to report that root microsomes from different inbreds and hybrids may contain one, two, or three of the total of five isoenzymes found among all of the lines tested.

MATERIALS AND METHODS

Corn (*Zea mays* L.) RNase II was prepared as previously reported (13, 14). Seed for each sample came from a single ear. Roots from 50 to 100 4-day-old seedlings (yielding 3 to 10 g fresh wt) were homogenized in two times their weight of 20 mM Tris-HCl buffer (pH 7.5). Large particles and cell debris were removed by centrifugation at 35,000g for 20 min and crude microsomal pellets were precipitated by centrifugation at 105,000g for 2 hr. The pellets were suspended in 1 to 2 ml of sample buffer composed of 15 mM citric acid, 42 mM HEPES, and 10% sucrose adjusted to pH 5.5 with NaOH. After centrifugation at 10,000g for 15 min, the supernatant solutions were used as the source of RNase II. The enzyme activity was stable for a year when the solutions were frozen. The final microsomal extract usually contained about 10% of the total activity of the original homogenate, with little contamination by either RNase I or nuclease I. Satisfactory enzyme activity on the gels required the extract from the equivalent of two to four seedlings.

Polyacrylamide disc gel electrophoresis was performed by the method of Davis (4), with only slight modifications. The spacer gel contained 0.062 M Tris adjusted to pH 6.7 with citric acid, 3% acrylamide, and 0.75% bis-acrylamide. The separating gel contained 0.375 M Tris adjusted to pH 8.9 with HCl, 7% acrylamide, and 0.184% bis-acrylamide. The electrode buffer was 0.01 M Tris with approximately 0.076 M glycine, adjusted to pH 8.3. The same number of bands were obtained with the pH 7.5 buffer previously used for RNase studies (14), but the resolution of RNase II was better with the pH 8.9 gel. From 30 to 100 μl of sample was layered on the gel, depending upon the enzyme activity of each sample. Electrophoresis was carried out for 15 min at 2 mamp/tube, then for 50 min at 4 mamp/tube, about 15 min past the time the bromphenol blue dye front ran off the gel. The RNase bands were revealed by incubating the gels in a buffered yeast RNA solution followed by a 30-sec wash with toluidine blue, as previously described (14).

An endosperm extract from R802 opaque-2 endosperm (766 units/g), diluted 1:20 with sample buffer, provided (in 10 μl) about 0.1 standard unit (16) of RNase I activity, against which the mobility of the RNase II bands were compared (R_I = [mm traveled by RNase II ÷ mm traveled by RNase I] × 100).

RESULTS AND DISCUSSION

The locations of the five RNase II isoenzymes in the gel are shown in Figure 1A, and the same isoenzymes plus an RNase I marker are shown in Figure 1B. Isoenzyme 1, the most rapidly moving, occurs in the inbred W64A (Fig. 1, gel 1) and in the inbreds Oh7N, WF9, R801, and B14A (not shown). Isoenzyme 2 was found in the inbred M14 (gel 2) and isoenzyme 3 in R803 (gel 3). The inbreds Oh43 (gel 4) and Oh45B (not shown) contained both isoenzymes 2 and 3, with the latter in higher amounts. N28 was the only inbred with prominent bands of isoenzymes 4 and 5 (gel 5), with band 5 being more intense. Trace amounts of activity at the isoenzyme 5 position occurred in some samples, especially after they had been stored, as for those shown in Figure 1. This might be an artifact, except in N28.

The various hybrids that were tested contained band patterns predictable from the parental patterns in all cases (Table I), including the relative proportions of isoenzymes 2 and 3 when Oh43 was a parent and isoenzymes 4 and 5 when N28 was a parent. Where single parental isoenzymes were involved (R801 × W64A, WF9 × M14, and R803 × M14), the hybrid pattern was that expected if these isoenzymes were variants of a single enzyme controlled by multiple alleles at one locus. The opaque-2 version of N28, derived from an unknown opaque-2 source and backcrossed by N28 several times, had band 1 in addition to bands 4 and 5 (Table I). W64A, which contained isoenzyme 1, is a common source of the opaque-2 gene.

The RNase II molecule is unlikely to consist of two or more subunits because of its small size. The crosses involving Oh43 and N28, however, contained the same double bands as the inbreds. The inbreds might be heterozygous for the RNase II allele, but it
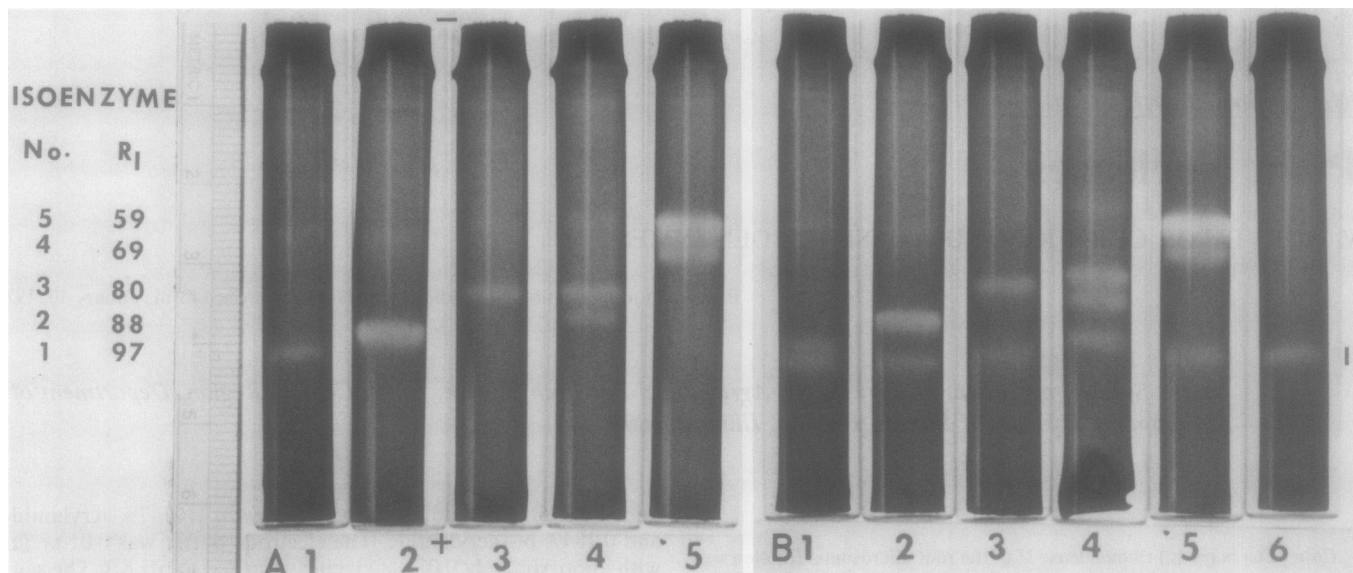
FIG. 1. Polyacrylamide gel electrophoresis of corn RNase II isoenzymes from corn inbreds. A: microsomal preparations only. B: microsomal preparations mixed with an endosperm extract containing RNase I. Gel 1, W64A; gel 2, M14; gel 3, R803; gel 4, Oh43; gel 5, N28; gel 6, endosperm extract. $R_I = 100$ for RNase I.

Table I. Occurrence and relative intensities of RNase II isoenzymes in corn hybrids.

Hybrid	Parent Isoenzymes		Isoenzymes in Hybrids
	Seed	Pollen	
R801 x W64A	1	1	1
WF9 x M14	1	2	1 = 2
R803 x M14	3	2	2 = 3
N28 x Oh7N	5 >> 4	1	1 = 5 >> 4
N28 x M14	5 >> 4	2	2 = 5 > 4
Oh43 x M14	2 < 3	2	2 > 3
Oh7N x Oh43	1	2 < 3	1 > 2 < 3
N28o$_2$[1]	5 > 4	1?	1 = 5 >> 4

[1] Not a hybrid. Opaque-2 version produced by crossing N28 with an opaque-2 line, then backcrossing with N28 and selfing.

is unlikely that this heterozygosity would have carried through the single plant involved in these crosses in all five cases studied. An alternative explanation is that a second or duplicate gene also specifies an RNase which is located in the crude microsomal fraction. Further genetic analysis would be simplified by use of RNase II prepared from a single more mature plant that could yield enough enzyme for testing.

The relative band patterns of the various inbreds and hybrids were checked by running mixtures of extracts—within the limits of the system the banding patterns represented the sum of the individual samples (not shown). Isoenzyme 1 is the most common RNase II isoenzyme. It moves almost as fast as RNase I in this gel system (Fig. 1B, gel 1) and might not be detected in extracts which contain both enzymes in the relative amounts occurring in corn roots. If electrophoresis is to be used in physiological studies on the relative levels of the different RNases, selection of inbreds with isoenzymes 2 (M14) or 3 (R803) would be advantageous.

Only two other examples of multiple PAGE[1] bands of plant nucleases are known in which the enzyme was identified. Oleson et al. (10) found two enzyme bands for a tobacco nuclease. Kowalski et al. (7) separated a highly purified mung bean nuclease into two active bands on PAGE. One band was made up of

enzyme molecules in which a single peptide bond had been cleaved (thus releasing an additional free carboxyl group), but which remained together by means of a sulfhydryl bond. Barley seedlings showed two bands in the gel with activity against RNA, but 4 out of 32 cultivars had a second pair of bands (2). Lontai et al. (8), however, detected seven polyuridylic acid-hydrolyzing enzymes in barley. van Loon (12) reported that tobacco leaves contained 17 bands of activity against polyuridylic acid. Four very active bands with exclusive RNase activity were charge isomers. RNase I is the most abundant ribonuclease in tobacco leaves (5), suggesting that isoenzymes of RNase I may exist in tobacco but not in corn. Most of the tobacco enzyme bands were also active against poly-d(a-T) and some had phosphodiesterase activity (12). Mejnartowicz and Bergmann (9), using starch gel electrophoresis, found a large number of RNase isoenzymes in conifer endosperm (haploid tissue), but the patterns of a single zone of bands appeared to be controlled by a distinct gene locus with two or three alleles. In Scots pine seed the isoenzyme variants were either a single band or a double band.

Caution should be used in interpretations of the presence of multiple RNase isoenzymes in crude plant extracts if the genetic background is not known. Corn hybrids may contain from one to three (and four would be expected in N28 × Oh43) RNase II isoenzymes, one form of RNase I, and three forms of nuclease I (14). In addition, one purified endosperm RNase I preparation included what is thought to be an artificially modified form of RNase I (14). Thus, bands of corn RNase activity separated by PAGE include: (a) genetically unrelated enzymes; (b) genetic variants; and (c) chemically modified or artificial variants. Studies on the roles of isoenzymes in development are most productive when the genetic background of the enzymes is known (11).

Corn RNases I and II are similar in their enzymic action, with both producing 2'3'-cyclic nucleotides (16). However, both can hydrolyze cyclic purine nucleotides but only RNase II can hydrolyze pyrimidine cyclic nucleotides. They also differ in pH optimum and in apparent location within the cell. RNase I shows no isoenzymes by use of PAGE (14), but it has a 5-fold range in activity that is genetically controlled in normal inbred endosperms. An additional 5-fold variation in activity occurs in the opaque-2 versions of some inbreds (15). The present study revealed five different isoenzymes of RNase II in root microsomal preparations from inbreds containing common endosperm RNase I. It is inter-

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[1] Abbreviation: PAGE: polyacrylamide disc gel electrophoresis.

esting that two superficially similar enzymes are controlled in different fashions. Johnson (6) has suggested that "in a heterogeneous environment the optimal evolutionary strategy for regulatory reactions is to have alternative enzyme forms available, rather than a single form of high capacity." Thus, it may be suggested that RNase II has a regulatory role in the growth and development of corn roots. An RNase II-like enzyme was reported to increase in ribosomes from illuminated lupin shoots (1) and in microsomes from auxin-treated pea stems (3). RNase I appears in apparent overabundance in developing corn endosperms and may not have a regulatory role. Also, the developing endosperm is better shielded from changing environments than are leaves and roots.

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