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THE PROTEIN SYNTHESIS INHIBITORS FROM WHEAT, BARLEY, AND RYE HAVE IDENTICAL ANTIGENIC DETERMINANTS

Richard R. Reisbig and Øyvind Bruland

Norsk Hydro's Institute for Cancer Research and
Norwegian Cancer Society, Det Norske Radiumhospital,
Montebello, Oslo 3, Norway

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SUMMARY: We have purified the protein synthesis inhibitors from wheat (tritin) and investigated their antigenic relationship to inhibitors from other grains and more distantly related plants. Three forms of tritin (tritin 1, 2, and 3) indistinguishable with regards to their molecular weight and enzymatic properties were found. Antiserum against the most abundant tritin, tritin 2, cross reacted with tritin 1, tritin 3, and with the translation inhibitors from barley and rye, but not with other plant-derived translational inhibitors. The results indicate that only closely related plants contain antigenically similar protein synthesis inhibitors.

In a wide variety of plants, there are proteins that can inhibit protein synthesis in cell free systems, by enzymaticly inactivating the 60s ribosomal subunit. Examples of these translation inhibitors include gelonin from Gelonium multiflorum (1) and dianthin 30 and 32 from Dianthus caryophyllus (2, 3), among others. Recently, Coleman and Roberts (4) have shown that the members of the Gramineae family, barley (Hordeum vulgare), rye (Secale cereale), oats (Avena sativa) and corn (Zea mays), also contain inhibitors, similar but not identical to the one from wheat germ, tritin (5).

Remarkably, many of these non-toxic inhibitors, from quite distantly related plants, have similar physical and enzymatic properties that are analogous to the A-chains of the well known toxins abrin and ricin (6). They all exist as basic monomeric proteins in neutral solutions, having in most cases, a molecular weight of ~ 30,000, and are quite resistant to proteases.

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These similarities indicate a close structural relationship between these inhibitors. To investigate this relationship, we studied the ability of these inhibitors, from many different plants, to antigentically cross react. We found that only the closely related plants wheat, barley, and rye were able to cross react, whereas unrelated translation inhibitors showed no antigenic cross reactivity. During this investigation, we developed a simple purification procedure for tritin that separated three distinct forms, not one as previously described (5).

METHODS

Materials: TSK-125 gel filtration column was a product from Bio-Rad. Wheat (Triticum aestivum), rye (Secale cereale), barley (Hordeum vulgare), rice (Oryza sativa), corn (Zea mays) and buckwheat (Fagopyrum esculum) were obtained from a local market.

Purification of tritins: Extracts of whole wheat were prepared by the method described by Roberts and Steward (5) and then dialyzed extensively against 5 mM sodium phosphate, pH 6.5. The extract was centrifuged for 15 min at 12,000 xg. The supernatant was collected and put through a carboxymethyl cellulose (CM-52) column (1.6x15 cm) as described in the legend to Fig. 1. Fractions inhibiting protein synthesis in reticulocyte lysates were pooled and then concentrated using CM-52 chromatography as follows: the pooled fractions were extensively dialyzed against 5 mM sodium phosphate, pH 6.5, and layered on a CM-52 column (0.6x1 cm), previously equilibrated in the same buffer. The column was washed with 20 mls of 5 mM sodium phosphate, pH 6.5, and the proteins were eluted with 200 mM NaCl, 5mM sodium phosphate, pH 6.5. Protein containing fractions were stored at -20°C.

Antisera, immunodiffusion tests and immunoprecipitation: Antisera to tritin 2 and dianthin 30 were produced as described (7). Two dimensional immunodiffusion was done on 1% agarose plates in 0.3 M NaCl, 10 mM phosphate pH 7.3. After incubation in a humidified box for 3 days at room temperature, the agarose plates were washed at 4°C for 3 days with 0.5 M NaCl and then for 2 days with distilled water. The gels were stained with Comassie blue and dried. Immunoprecipitations were carried out according to the method of Ivarie and Jones (8).

Other procedures: ^{125}I labelling of proteins was perfomed as described by Fraker and Speck (9). Sodium docecyl sulfate (SDS) gel electrophoresis was run as described by Olsnes and Eiklid (10). Protein concentrations were determined by the method of Lowry (11) using human γ-globulin as a standard. The purification of dianthin 30 and 32, the purification of IgG fraction of antiserum, protein synthesis in reticulocyte lysates, and gel filtration HPLC were carried out as described elsewhere (3).

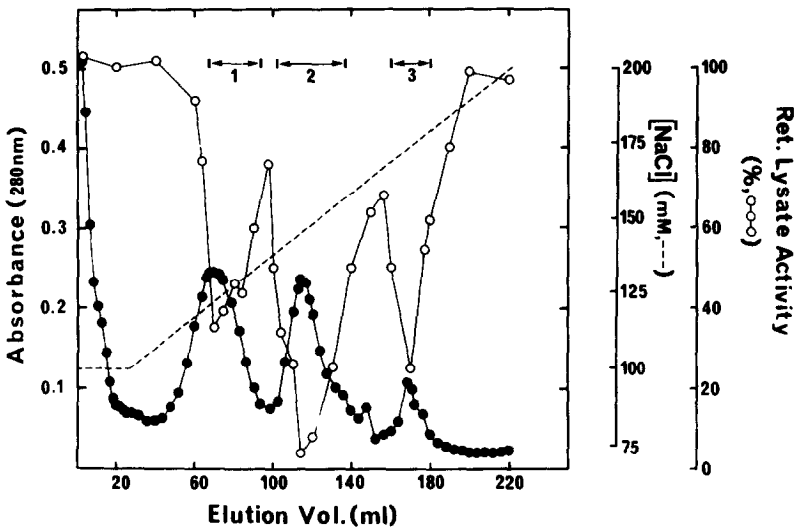

Fig. 1. Purification of three forms of tritin on a carboxymethyl cellulose column. Whole wheat extracts were run on a CM-52 column as described in the text. The column was first washed with 100 mls of 5mM phosphate, pH 6.5 and then with 100 mls of 100 mM NaCl, 5 mM phosphate, pH 6.5. A 220 ml linear gradient from 100 mM to 200 mM NaCl in 5 mM phosphate pH 6.5 was then applied to the column. The absorbance at 280 nm (●—●) was measured. After a ten fold dilution the ability of the fractions to inhibit protein synthesis in reticulocyte lysates (O—O) was determined as described in "Methods".

RESULTS AND DISCUSSION

Based on the purification schemes for gelonin and dianthin 30 (1, 2) we have developed a simple one-step procedure for purifying the tritins from wheat.

Extracts of whole wheat were layered on a CM-52 column and after first washing with low salt and then with a buffer containing 100 mM NaCl, the tritins were eluted by a gradient from 100 mM NaCl to 200 mM NaCl. Three peaks, each having the ability to inhibit protein synthesis in reticulocyte lysates, were obtained (fig. 1).

Peak 2, eluting at 140 mM NaCl (tritin 2), and peak 3, eluting at 170 mM NaCl (tritin 3), contained one polypeptide having a molecular weight of 30,000 (fig. 2); a value very close to the one previously determined for tritin (6). Peak 1 eluting at 125 mM NaCl (tritin 1), contained a number of commassie blue

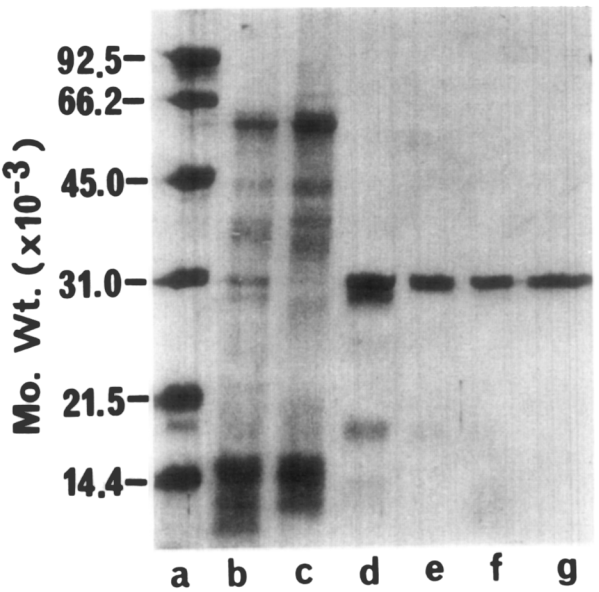

Fig. 2. SDS polyacrylamide gel electrophoresis of the various steps during the purification of the tritins. Molecular weight markers (a), 10 ug of whole wheat extract (b), 15 ug of the proteins not binding to the CM-52 column (c), 6 ug of protein from peak 2 in fig. 1 (d), 2 ug of protein from peak 2 in fig. 1 (e), 2 ug of protein from peak 3 in fig. 1 (f), and 2 ug of protein from the peak eluting at 8.45 min. from the gel filtration HPLC (g).

staining bands, and thus, was further purified by running the concentrated fractions on gel filtration HPLC. A peak eluting from the column at 8.45 min. was able to inhibit protein synthesis in reticulocyte lysates and contained one polypeptide with a molecular weight of 30,000 (fig. 2 g), identical to that of tritin 2 and 3. Similar results to those presented above were obtained when the protease inhibitor phenylmethyl sulfonyl fluoride (PMSF, 10 ug/ml) was included in all the buffers during the purification procedure.

The three forms of tritin had very similar chemical and enzymatic properties. All three were shown to be monomers in solution, eluting from a gel filtration column (HPLC) at a time corresponding to a molecular weight of 29,300 (data not shown). Tritin 1, 2 and 3 displayed almost identical absorption spectra

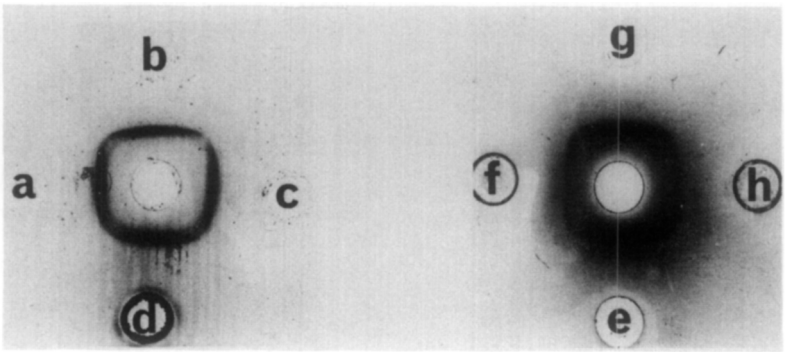

Fig. 3. Double diffusion immunoprecipitation of the tritins and grain extracts with anti-tritin 2. The wells contained 10 ul of (a) tritin 2 (0.5 mg/ml), (b) tritin 1 (0.2 mg/ml), (c) tritin 3 (0.3 mg/ml), (d) wheat extract (5 mg/ml), (e) tritin 2 (0.5 mg/ml), (f) wheat extract (5 mg/ml), (g) rye extract (3mg/ml), and (h) barley extract (5mg/ml). The center wells contained 10 ul of tritin 2 antiserum.

in the range of 200 nm to 320 nm and had similar specific activities with respect to inhibition of protein synthesis in reticulocyte lysates (50% inhibition required 250 ng/ml of tritin 1, 2, or 3). They all were resistant to protease activity (data not shown). All three had similar electrophoretic mobilities on nondenaturing gels analysed at pH 8.3. However, on gels at pH 3.5, the tritins showed small but reproducible differences in electrophoretic mobility (data not shown).

These results suggest that there are at least three similar forms of tritin (izoenzymes) in whole wheat not one as previously described (5). We have also purified three forms of the translation inhibitors from barley by the same method as described here for wheat.

To analyse the antigenic relationship between the different tritins and other translation inhibitors, we used the double immunodiffusion technique developed by Ouchterlony (12). Tritin 2 antiserum cross reacted with tritin 1 and 3 and with whole wheat extracts (fig 3). Each precipitation reaction was continuous with the reaction next to it, indicating that all three tritins have identical antigenic determinants. Similar results were observed

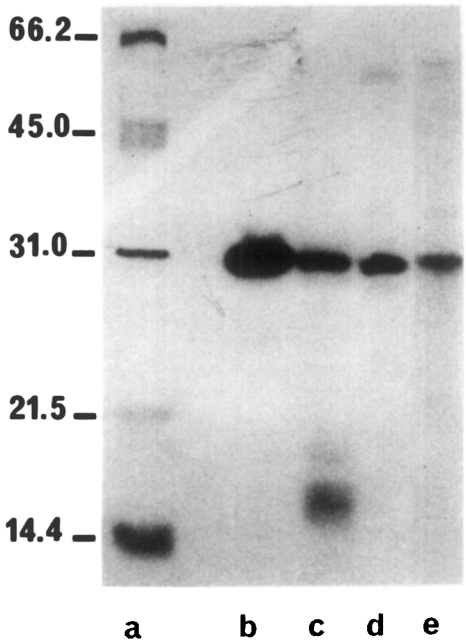

Fig. 4. Autoradiograph of an SDS electrophoresis gel of proteins immunoprecipitated with antitritin 2 from ^{125}I labeled extracts of wheat, barley, and rye. Tritin 2 (b) or protein extracts from wheat (e), barley (d), or rye (e).

when extracts of barley and rye were tested with anti-tritin 2 in the double diffusion reactions (fig.3).

Since the translation inhibitors from barley and rye are very similar to tritin (4), it seemed likely that the inhibitors in these grain extracts were cross reacting with anti-tritin 2. In agreement with this possibility, we found that anti-tritin 2 reduced by over 95% the ability of the grain extracts to inhibit protein synthesis in reticulocyte lysates. When ^{125}I labeled protein extracts were immunoprecipitated with anti-tritin 2, one major protein having an identical electrophoretic mobility to tritin 2 was found for wheat, barley, and rye (fig. 4). These results indicate that the translation inhibitors of the closely related grains, rye, barley and wheat have identical antigenic determinants.

On the other hand, oats (Avena sativa), a somewhat distantly related member of the Graminae family, showed no cross reactivity

when protein extracts were tested with anti-tritin 2. Neither did corn (Zea mays), rice (Oryza sativa), nor buckwheat (Fagopyrum esculum) form precipitin bands. Like Falasca et al. (13), we found no cross reactivity between gelonin and dianthin 30. We also found that the tritins, dianthins, or ricin A chain had no cross reacting antigentic determinants.

Our results indicate that the translation inhibitors from closely related plants are antigenically similar, while those from more distantly related plants are dissimilar. This strongly suggests that the structure of these translation inhibitors has not been highly conserved in the higher plants.

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