Note on the Identical Immunological Behavior of a Protein Fraction from Durum Wheat Germ and a Purified Lectin from Soft Wheat Germ

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A method was recently described (Pompucci et al 1978) for the purification from durum wheat germ (*Triticum durum*) of a protein fraction with a molecular weight of 40,000. A specific antiserum against this fraction (DWG fraction) has also been prepared, to be used as an immunochemical method for the determination of wheat germ added to pasta products (Cantagalli et al 1979). Lectin from soft wheat (*Triticum aestivum*) germ (SWG), on the other hand, is well known. It has been studied for its hemoagglutinating properties and more specifically for its tendency to bind to receptors on the cell membranes (Aub et al 1963).

In this study, we investigated the immunological pattern developed by the antiserum against DWG fraction, assayed by double immunodiffusion in agarose gel with purified SWG lectin (Allen et al 1973), soybean lectin (Lis et al 1966), and protein extracts from soft wheat flour and durum wheat semolina, both free from wheat germ. Our objectives were: 1) to demonstrate the immunological identity between SWG lectin and the DWG fraction and 2) to determine whether the antibody against the DWG fraction reacts with soybean lectin or with durum or soft wheat protein other than lectin.

MATERIALS AND METHODS

Materials

SWG lectin and lectin from soybean were obtained from Pharmacia, Uppsala, Sweden. Soft and durum wheats, obtained from a commercial source, were ground using a laboratory grinder (Bühler, Milan, Italy). Durum wheat germ was supplied by the Agnesi pasta company (Imperia, Italy).

Extraction of Proteins

Soluble proteins were extracted at 5°C for 15 hr by mixing 2 g of ground wheat with 4 ml of 0.15*M* NaCl containing 0.1% NaN_3. After centrifugation at 10,000 × *g* for 15 min, the supernatant was used for protein analysis.

Purification of DWG Fraction and Immunization

The DWG fraction was prepared according to Pompucci et al (1978). Durum wheat germ was defatted in a Soxhlet apparatus and extracted twice with distilled water for 24 hr at 4-5°C. After centrifugation at 30,000 × *g*, the clear supernatant was injected into rabbits to produce an immune serum.

An additional extract was prepared in the same way and fractionated by precipitation with ammonium sulphate. The various fractions were tested against the anti-durum wheat germ immune serum. The fraction that was precipitated with 25-35% ammonium sulphate showed the greatest immunoreaction. This fraction was chromatographed on a Sephadex G-200 column, using a tris-HCl 0.1*M* buffer, pH 7.4, containing 0.1*M* NaCl.

The only fractions giving an immunological reaction were those with a molecular weight of about 40,000. An antiserum against the DWG fraction was prepared with these fractions, following the method proposed by Cantagalli et al (1979). An antiserum against SWG lectin was prepared by injecting rabbits with a solution of purified lectin at a concentration of 1.4 mg/ml in 0.15*M* NaCl and 0.01% thimerosal. Each rabbit received 1.4 mg of antigen by subcutaneous route, in two injections given 40 days apart. The first injection was given in Freund's complete adjuvant, the second in incomplete adjuvant. The animals were bled 20 days after the last treatment.

Immunochemical Methods

Double immunodiffusion in agarose gel and dyeing were performed according to Outcherlony (1968). The analysis at various ratios of reactants was carried out according to Piazzi (1969).

RESULTS AND DISCUSSION

The double immunodiffusion between the anti-DWG fraction serum, SWG lectin, and the DWG fraction showed a pattern of identity (Fig. 1). In the central area, both precipitation lines completely fused. In balanced systems, this arc formed by the fusing lines is symmetrical. The reaction indicates the presence of identical antigenic determinants in the reactants under study.

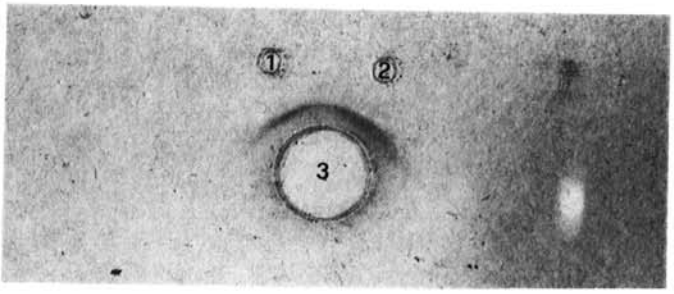

Fig. 1. Double immunodiffusion analysis. The wells contained: **1,** soft wheat germ lectin, 5 μl (1.4 mg/ml); **2,** durum wheat germ fraction, 5 μl (1.7 mg/ml); **3,** antiserum against durum wheat germ fraction, 60 μl.

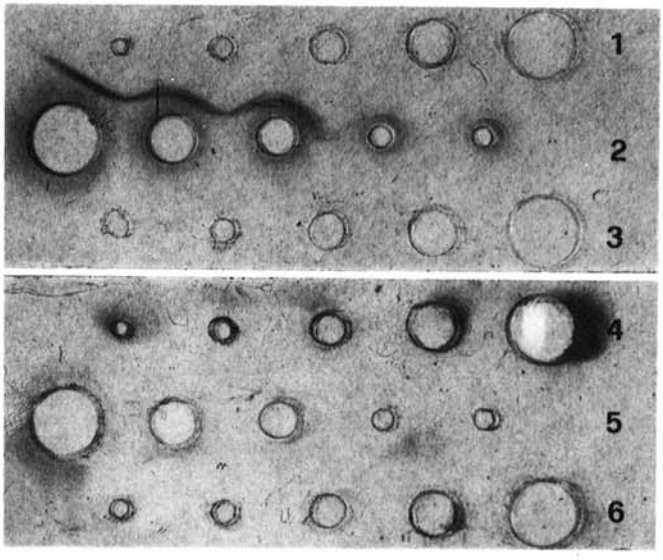

Fig. 2. Double immunodiffusion analysis at different volumes of reactants. The wells contained: row **1,** durum wheat germ fraction (1.6 mg/ml); rows **2** and **5,** antiserum against durum wheat germ fraction; row **3,** soybean lectin (3 mg/ml); row **4,** semolina protein extract (6 mg/ml); row **6,** flour extract (6 mg/ml).

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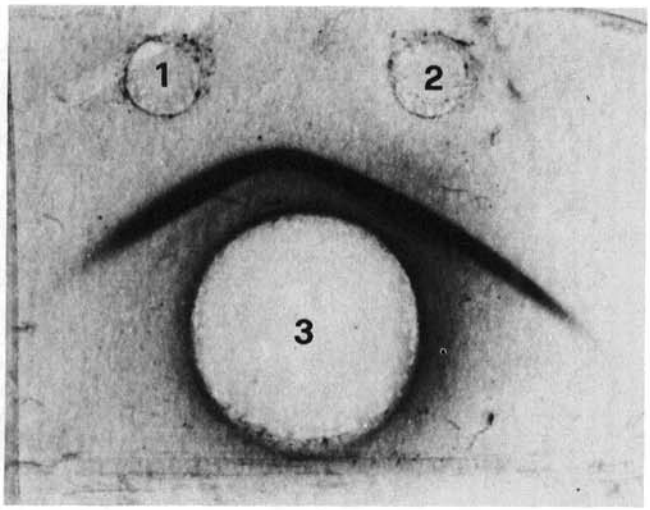

Fig. 3. Double immunodiffusion analysis. The wells contained: **1,** durum wheat germ fraction, 5 μl (1.7 mg/ml); **2,** lectin from soft wheat, 5 μl (1.4 mg/ml); **3,** antiserum against soft wheat lectin, 60 μl.

No precipitation was detected with soybean lectin and flour and with semolina extracts when assayed by double immunodiffusion at various ratios of reactants (Fig. 2). The double immunodiffusion analysis performed as shown in Fig. 2, allows us to establish with a single test the conditions in which the formation of immunoprecipitate is possible. In fact, in double immunodiffusion, precipitation lines only form when antigen and antibody are present in the same concentrations. In the present case, the antibody (anti-DWG fraction) in the wells of rows 2 and 5 showed the greatest immunoreactivity with the antigen (DWG fraction) in the wells of row 1 at the place where the antibody well was of maximum diameter and the antigen well was of minimum diameter. The reaction became weaker as the concentrations of antigen and antibody varied, until it disappeared completely. No immunoprecipitate formed with the antigens in the wells of rows 3, 4, and 6. We can therefore conclude that the antiserum is specific for the DWG fraction.

The anti-SWG lectin serum showed the same properties as the anti-DWG fraction serum, developing a pattern of identity between the immunizing antigen and the DWG fraction when tested in double immunodiffusion (Fig. 3).

Our results indicate that the fraction with molecular weight 40,000 obtained from durum wheat germ shows an immunological pattern of identity with lectin from soft wheat germ. This identity pattern was confirmed by using both an antiserum against the DWG fraction and an antiserum against commercial SWG lectin. Our findings lead us to consider the DWG fraction isolated by Pompucci et al (1978) as a lectin from durum wheat germ. Furthermore, we were unable to detect any immunological differences between soft wheat lectin and durum wheat lectin.

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