Lipid-Mediated Aggregation of Gliadin[1]

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ABSTRACT

Gliadins from undefatted and completely defatted flour were fractionated by gel filtration chromatography on a Sephadex G-200 column. Fraction I (excluded) from undefatted flour contained almost all of the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) subunits of the original unfractionated gliadin. After removal of lipids from this fraction, its protein was resolved into five peaks (fractions) on rechromatography on Sephadex G-200. The five protein fractions of the gliadin from defatted flour were reconstituted with the original lipids and chromatographed on Sephadex G-200. SDS-PAGE showed that the subunit composition of fraction I was similar to that of the original gliadin from undefatted flour. It was concluded that the lipid-mediated aggregation of high and low molecular weight subunits of gliadin is reversible.

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Our previous work (Békés et al 1983) showed that the ethanol-soluble fraction of gluten (mainly gliadin) prepared from undefatted flour contained about 10% nonprotein material, which was identified as lipid (mainly galactolipid). Furthermore, it was shown that the protein composition by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of fraction I (obtained by gel filtration on Sephadex G-200) depended on whether or not lipids were present in the original gliadin preparation. Experiments were therefore performed to determine whether lipids were involved directly in the aggregation of specific gliadin proteins.

MATERIALS AND METHODS

The two "gliadin" preparations used in this study were prepared from gluten washed from undefatted and totally defatted (by extraction with water-saturated [WSB] *n*-butanol followed by hexane) flour milled from hard red spring wheat cultivar Neepawa (Békés et al 1983). The preparations are the same as gliadins F and L, discussed in our previous article (Békés et al 1983). The sample from the undefatted flour contained 8.95% lipid (2.93% free and 6.02% bound; 3.76% nonpolar and 5.19% polar) (Békés et al 1983). The sample from defatted flour contained no lipid.

AU solvent (0.1*N* acetic acid and 3*M* urea) was used in all chromatography experiments. Fraction I of the two gliadins (eluted in the void volume on Sephadex G-200) was collected. A portion of each fraction I was concentrated by vacuum evaporation to 40° C under nitrogen and the resulting solution rechromatographed on Sephadex G-200. The remainder of each fraction I was dialyzed and freeze-dried. An appropriate portion of the dry material was dissolved in AU solvent and rechromatographed; the remainder was extracted with WSB and rechromatographed.

Reconstitution of the protein fractions of the gliadin from defatted flour and the WSB-soluble lipids of the gliadin preparation from undefatted flour was made to equal the composition of the gliadin preparation from undefatted flour. Accordingly, 20 mg of fraction I, 10 mg of fraction II, 50 mg of fraction III, 14 mg of fraction IV, and 6 mg of fraction V, were dissolved in 100 ml of 70% aqueous ethanol and mixed with 10 ml of 70% ethanol solution containing 10 mg of the WSB-soluble lipids. The added lipids comprised 4.20 mg nonpolar, 4.86 mg glycolipid, and 0.94 mg phospholipid (Békés et al 1983). The alcoholic mixture was stirred gently at room temperature for 5 hr. The solvent was then removed by nitrogen aeration at 40° C and the residue dissolved in 10 ml AU solvent. Five milliliters of the resulting solution was chromatographed on Sephadex G-200. Fraction I (excluded) was collected, dialyzed against distilled water, concentrated by nitrogen aeration, and rechromatographed. The rechromatographed fraction I was subjected to analysis by SDS-PAGE by the procedure of Khan and Bushuk (1977).

RESULTS AND DISCUSSION

Figure 1 (A,B) shows that both gliadin preparations (with and without lipid) gave five protein peaks on gel filtration chromatography on Sephadex G-200. A major difference between

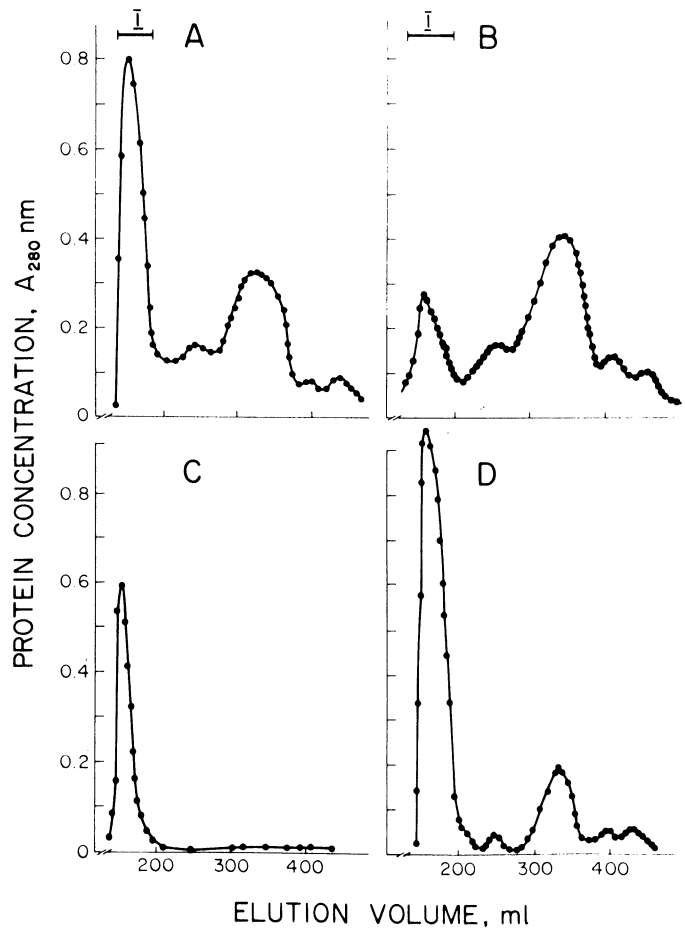

Fig. 1. Elution profiles of gliadin preparations and fractions thereof: **A,** gliadin from original (undefatted) flour; **B,** gliadin from defatted flour; **C,** rechromatography of fraction I (see A) of gliadin from undefatted flour; and **D,** rechromatography of freeze-dried and defatted fraction I of gliadin from undefatted flour.

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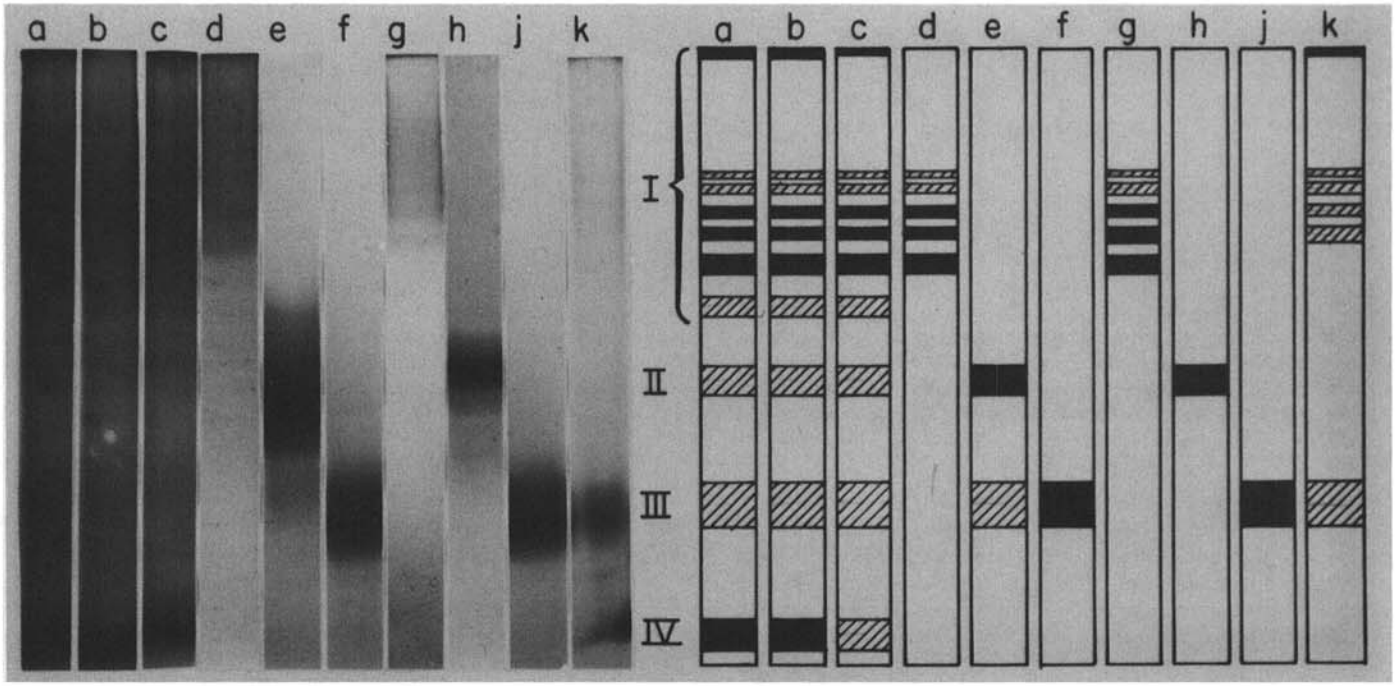

Fig. 2. SDS-PAGE patterns of gliadin fractions prepared by gel filtration chromatography on Sephadex G-200: **a,** fraction I of gliadin from undefatted flour; **b,** fraction I from rechromatography of fraction I of gliadin from undefatted flour after removal of solvent by evaporation; **c,** fraction I from rechromatography of fraction I of gliadin from undefatted flour after freeze-drying; **d,** fraction I from rechromatography of defatted fraction I of gliadin from undefatted flour; **e,** fraction II from rechromatography of defatted fraction I of gliadin from undefatted flour; **f,** fraction III from rechromatography of defatted fraction I of gliadin from undefatted flour; **g,** fraction I of gliadin from defatted flour; **h,** fraction II of gliadin from defatted flour; **j,** fraction III of gliadin from defatted flour; and **k,** fraction I of reconstituted gliadin preparation.

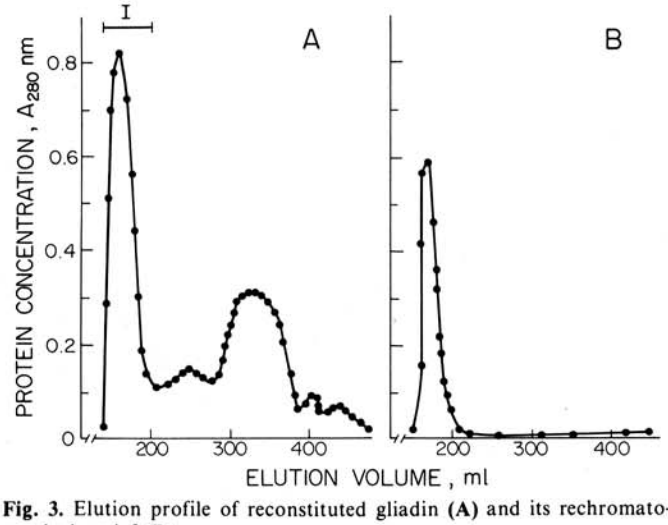

Fig. 3. Elution profile of reconstituted gliadin **(A)** and its rechromatographed peak I **(B)**.

the two preparations was in the amount of fraction I; the preparation from defatted flour contained less fraction I (47% for the undefatted preparation vs 19% for the other). The relative proportions, by weight, of the other four fractions were 4, 32, 10, and 7%, and 8, 51, 12, and 10% for undefatted and defatted preparations, respectively.

Rechromatography of fraction I produced a symmetrical peak, again in the void volume. After concentration by evaporation (not shown) and freeze-drying (Fig. 1C) and rechromatography, fraction I did not contain any of the lower mol wt fractions. The SDS-PAGE subunit compositions of the rechromatographed fraction I before and after freeze-drying were qualitatively identical (Fig. 2b,c).

After defatting, fraction I gave a chromatographic profile similar to that of the original gliadin (compare Figs. 1D and 1A). SDS-PAGE patterns (Fig. 2) of the separated fractions were identical to the equivalent fractions of the original gliadin from completely defatted flour (compare d and g, e and h, and f and j). These results indicate that defatting produced a disaggregation of low and high mol wt subunits.

Reconstitution of the lipids to protein fractions of gliadin from defatted flour reversed the aggregation of low mol wt subunits into fraction I. Rechromatographed fraction I of reconstituted gliadin gave a single peak (Fig. 3B); the SDS-PAGE pattern of this fraction (Fig. 2k) contained the major low mol wt subunit in addition to the high mol wt subunits.

It is therefore concluded that the lipids extracted with the gliadin proteins by 70% aqueous ethanol solution can mediate the aggregation of some gliadin subunits to form aggregates of high mol wt. Additionally, it is postulated that this lipid-mediated aggregation of gliadin is relevant to the functionality of this protein in breadmaking.

LITERATURE CITED

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