Petroleum Ether-Soluble Lipoprotein of Barley Flour[1]

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ABSTRACT

Water-saturated butanol precipitated a protein fraction from the petroleum ether-soluble oil of barley flour. The protein fraction (0.84% of the total free oil) contained 45.0% protein and 24% carbohydrate. The protein migrated as a doublet during starch-gel electrophoresis. The lipoprotein contained relatively high amounts of the amino acids lysine, arginine, and cysteine. The protein isolated from barley flour oil appeared to have properties that were similar to those of the protein fractions isolated from wheat flour oil.

The presence of protein in petroleum-ether extracts of wheat flour was first reported by Balls and Hale (1). They isolated the protein called purothionin as a crystalline hydrochloride, and found that it had an unusual amino acid composition (2).

Fisher et al. (3) fractionated purothionin hydrochloride into two fractions by gel filtration on Sephadex. The molecular weights of the two fractions were estimated at 45,000 and 10,000, respectively. Electrophoretically the lower molecular-weight fraction corresponded to the "fast-moving globulin doublet" of wheat flour. The similarity of the "fast-moving globulin" and purothionin was stressed by Nimmo et al. (4). Their globulin preparation resembled purothionin in amino acid composition, electrophoretic mobility at pH 3.1 and 8.5, ultraviolet spectra, and gel-filtration properties. Additional evidence of the close similarity between purothionin and globulin was reported by Redman and Fisher (5). They found purothionin and the globulin "fast-moving doublet" to have the same C-terminal end group, the same peptide "fingerprint" after trypsin digestion, and similar, but not identical, amino acid composition.

Quantitative isolation, by a relatively simple solvent system, of the two protein fractions present in the petroleum ether-soluble oil of wheat flour was reported by Hoseney et al. (6). One protein fraction (about 0.7% of the total free lipids) contained 60% protein and 35% carbohydrate. The second protein fraction (22% of the total free lipids) contained 30% protein, 7.5% carbohydrate, and about 62.5% lipids. Neither protein fraction had a significant role in breadmaking. In addition it

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was found that extracting flour with 6% sodium chloride yielded a globulin protein fraction containing 32.5% lipid. Proteins were detected in petroleum-ether extracts of that globulin fraction.

Redman and Fisher (5) reported that petroleum-ether extracts of barley flour contained components with electrophoretic properties that were similar to those of purothionin.

In a recent paper Redman and Fisher (7) reported the isolation of two proteins (termed hordothionin) from barley flour by acid treatment of a petroleum-ether extract. The two proteins were electrophoretically similar to the purothionin doublet obtained from wheat by the same procedure. Comparison of amino acid composition, peptide maps, immunological reactions, and C-terminal end groups confirmed a relation between the proteins isolated from barley and wheat. However, there were some differences in amino acid composition and sequence.

The purpose of our study was to isolate lipoprotein fractions from barley by the same solvent system that was used to isolate wheat lipoproteins, and to compare the petroleum ether-soluble proteins from barley and wheat.

MATERIALS AND METHODS

The oil used in this study was extracted from barley flour with petroleum ether (b.p. 35° to 60°C.) in a large Soxhlet. The barley flour (Larker variety grown at Fargo, N. Dak., in 1967) was experimentally milled at an extraction rate of 60.4%, and contained 1.04% ash, 9.1% protein, and 1.61% total free lipid (14% moisture basis). The petroleum-ether extracts were evaporated under vacuum and stored at 4°C. until used.

Starch-gel electrophoresis was performed in a vertical position with pH 3.2 aluminum lactate-lactic acid buffer containing 3.0M urea. Electrophoresis was performed at 30 ma. and approximately 325 v. for 4 hr. at room temperature. The gel was sliced and stained with 0.1% Amido Black 10B. Details of the procedure were given previously (8).

Thin-layer chromatography was performed on 100 γ of lipid. After they were developed with chloroform or chloroform-methanol-water (65:25:4), the plates were sprayed with a 70% (by volume) aqueous sulfuric acid that was saturated with $K_2Cr_2O_7$, charred at 150°C. for 30 min., and then photographed under ultraviolet light.

Paper chromatography was used to identify sugar residues following hydrolysis. The samples were hydrolyzed with 2N hydrochloric acid in an evacuated, sealed tube at 110°C. for 5 hr. The hydrolysate was evaporated to dryness over sodium hydroxide, adjusted to volume, and spotted on Whatman No. 4 paper. The chromatogram was developed in the long direction for 18 hr. with the top layer of a mixture of butanol-acetic acid-water (63:10:27). After drying, the paper was developed for 12 hr. in the short direction with a mixture of butanol-pyridine-benzene-water (5:3:1:3). Spots were visualized by dipping the chromatogram in a solution of silver nitrate in moist acetone, followed by dipping in dilute potassium hydroxide in methanol. The spots were fixed by dipping in an aqueous photographic fixer. Additional details of the procedure are given by Linko et al. (9).

Protein in lipid fractions was detected by a spot test. The lipid in petroleum

ether or 2:1 chloroform-methanol was spotted on filter paper, and the solvent was allowed to evaporate. The paper was dipped in a 0.1% aqueous solution of Amido Black 10B for 5 min. The paper was removed from the dye and washed repeatedly with distilled water. The presence of protein was indicated by a dark spot on a white background.

Amino acids were determined with a Beckman Model 120C. Twenty-five milligrams of sample was hydrolyzed with 6 ml. of 6.0N HCl in an evacuated, sealed tube for 20 hr. at 110°C. The hydrolysate was filtered (Whatman No. 4), evaporated to dryness over solid sodium hydroxide, and diluted to volume. Tryptophan was determined after hydrolysis with barium hydroxide essentially as described by Miller (10).

Protein was determined by the Kjeldahl procedure (11), and total carbohydrate by the phenol-sulfuric acid method (12).

RESULTS AND DISCUSSION

Isolation of Protein Fraction

The isolation procedure (Fig. 1) is similar to one previously used to isolate petroleum ether-soluble proteins from wheat flour oil (6).

The oil extracted from barley flour with petroleum ether (patterns 3 and 7, Fig. 2) contains much less polar lipids than the corresponding fraction from wheat flour (patterns 1 and 5, Fig. 2). The presence of protein in the petroleum ether-extracted oil from barley flour was confirmed by a spot test with aqueous Amido Black 10B on filter paper.

The petroleum ether-soluble oil was dissolved in water-saturated butanol (1 part oil to 10 parts butanol). The spontaneous precipitate, a white solid, was removed by filtration, washed with water-saturated butanol, and then extracted in a Goldfisch extractor with petroleum ether. The petroleum ether-solubles (principally sterols) contained no protein. The oil soluble in water-saturated butanol gave a negative protein spot test. In addition, the thin-layer chromatogram of the butanol-soluble oil (pattern 8, Fig. 2) shows only a light spot at the origin. The butanol-soluble oil from wheat flour (pattern 6, Fig. 2) does show a spot at the origin, presumably indicating the presence of lipoprotein. Thus, all the protein in barley lipids was precipitated with water-saturated butanol.

Characterization of Protein Fraction

The protein fraction (0.84% of the total free oil) contained 45.0% protein (N X 6.25) and about 24% carbohydrate. The remaining 31% is assumed to be mainly lipid. However, the lipid must be strongly bound since it is not extracted by water-saturated butanol. The protein fraction isolated from wheat flour oil by the same procedure contained no lipid, and thus had higher protein and carbohydrate contents than those of the protein fraction of barley oil.

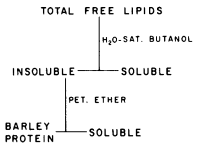

Fig. 1. Fractionation scheme for isolating a protein from total free barley flour lipids.

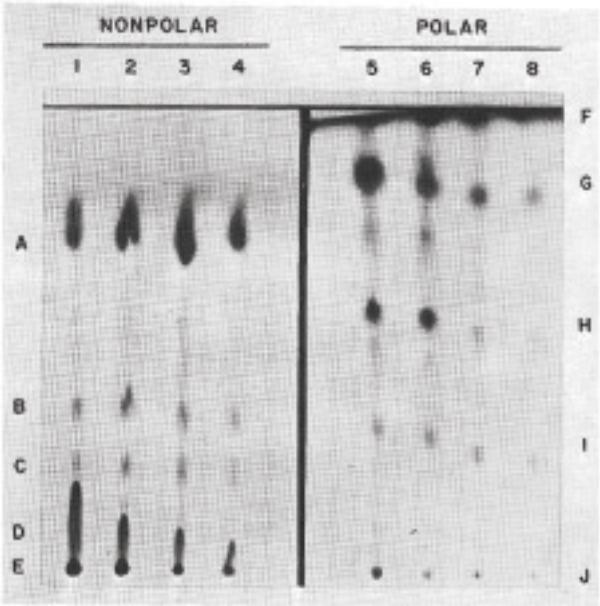

Fig. 2. Thin-layer chromatogram of: wheat total free lipids (patterns 1 and 5), wheat total free lipids soluble in water-saturated butanol (patterns 2 and 6), barley total free lipids (patterns 3 and 7), and barley total free lipids soluble in water-saturated butanol (patterns 4 and 8). Nonpolar lipids were developed with chloroform, and polar lipids were developed with chloroform-methanol-water (65:25:4). Spots are tentatively identified as follows: (A) hydrocarbons, steryl esters, and triglycerides, (B & C) diglycerides, (D) free fatty acids, (E) unresolved polar lipids, (F) unresolved nonpolar lipids, (G) monogalactosyl diglycerides, (H) digalactosyl diglycerides, (I) phosphatidyl choline, and (J) lipoprotein.

The starch-gel electrophoretic pattern of the barley protein fraction (Fig. 3, left) has one predominant doublet band and one extremely light band. Protein fraction I from wheat flour oil (middle pattern), isolated by the same procedure as the barley protein, has a much slower electrophoretic mobility than the barley protein. However, the barley protein's mobility is similar, but not identical, to the mobility of protein fraction II (right pattern) isolated from wheat flour oil.

The carbohydrate in the barley sample was identified by paper chromatography (Fig. 4) as glucose. Both protein fractions I and II isolated from wheat flour oil contained galactose and arabinose.

Amino acid analysis of the protein fraction isolated from barley oil (Table I) was characterized by relatively high values for lysine and arginine, and an extremely high value for cysteine. In general, the amino acid composition is similar to that of protein fraction II isolated from wheat flour oil (6). However, the barley protein is significantly higher in glutamic acid and phenylalanine.

Fisher et al. (3) reported that a purothionin preparation from wheat (fraction C) contained 0.7 g. tryptophan per 100 g. protein. However, purified purothionin preparations of Redman and Fisher (7) and Nimmo et al. (4) were reported to contain no tryptophan. Protein fraction II, isolated by us, corresponded electrophoretically to Fisher's fraction C, and contained 0.7 g. tryptophan per 100

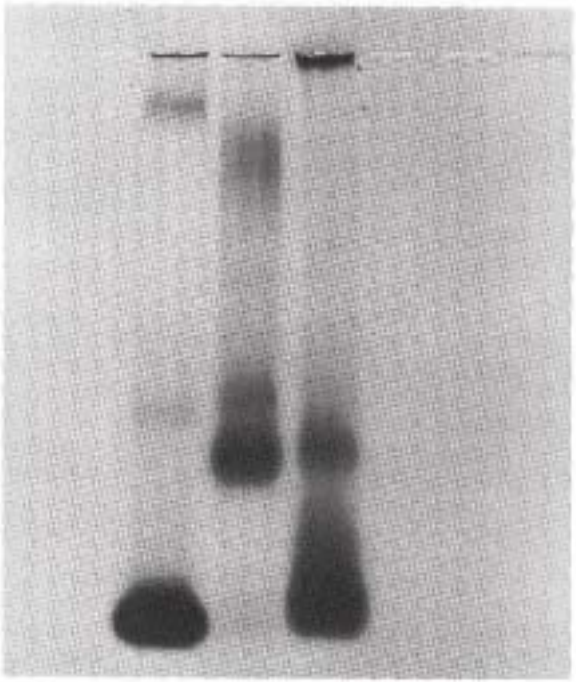

Fig. 3. Starch-gel electrophoretic patterns of barley lipoprotein (left), wheat protein fraction I (middle), and wheat protein fraction II (right).

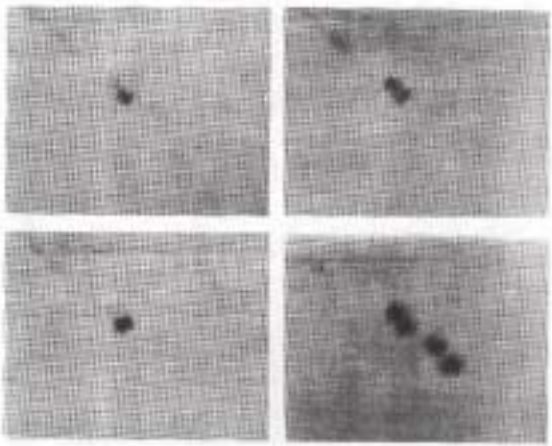

Fig. 4. Paper chromatograms of sugars in the hydrolysate of barley lipoprotein (upper left), hydrolysate of barley lipoprotein plus glucose (lower left), and hydrolysate of barley lipoprotein plus galactose (upper right). Standards (lower right, top to bottom) are galactose, glucose, arabinose, and xylose.

TABLE I. AMINO ACID COMPOSITION (g. AMINO ACID PER 100 g. PROTEIN) OF THE BARLEY LIPOPROTEIN FRACTION

Amino Acid	Barley Lipoprotein	Amino Acid	Barley Lipoprotein
Lysine	9.63	Alanine	4.05
Histidine	1.05	Cysteine	10.02
Ammonia	1.57	Valine	4.40
Arginine	10.16	Methionine	1.00
Aspartic acid	6.61	Isoleucine	2.39
Threonine	4.40	Leucine	7.94
Serine	5.81	Tyrosine	3.77
Glutamic acid	9.46	Phenylalanine	6.01
Proline	5.61	Tryptophan	0.90
Glycine	6.11	Total	100.84

g. protein. Protein fraction I from wheat flour oil contained 2.4 g. tryptophan per 100 g. protein, an extremely high value. The protein fraction isolated from barley oil contained 0.9 g. tryptophan per 100 g. protein. Redman and Fisher (7) reported that tryptophan was not detected in a highly purified barley protein (hordothionin α). The apparent disagreement can probably be explained by the possible loss of a high tryptophan entity during purification.

Several workers (3,4,5) have noted similarities between proteins isolated from free wheat flour oil and certain wheat flour globulins. Recently, we (6) reported that wheat globulins, extracted from flour with a 6% NaCl solution, contained 32.5% lipid. A spot test indicated lipoprotein in the oil extracted by petroleum ether from the globulin fraction. Therefore, barley flour was extracted with a 6% sodium chloride solution (250 g. flour per 600 ml. solution). The extract was dialyzed against distilled water and centrifuged to recover the precipitated barley globulins. They yielded 16% lipid when extracted with petroleum ether. A spot test, however, indicated that the lipid contained no protein.

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