Two Protein Fractions of Barley, Precipitated from Acid Solutions

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When water extracts of barley or malt (pH 5.8–8.0, +20° C, 0.1 % sodium bromate present) are acidified to pH 4.5–4.0, a brownish protein precipitate is obtained, designated in the following as fraction I. A white protein, called fraction II, is precipitated from the supernatant by addition of sodium chloride to a concentration of 2 moles per liter. When prepared from cold, clear-filtered extracts, these precipitates are almost completely redissolvable at pH 7.

Both these fractions are precipitated by ammonium sulphate in the saturation range 15–40 % (at pH 6.0), but the precipitates obtained in this way are largely insoluble. Thus, it is possible that a part of the proteins in these fractions has escaped the attention of numerous investigators of barley proteins. It may be mentioned, that Quensel¹ discarded the globulin fraction precipitated by 15–40 % saturated ammonium sulphate (at pH 7.0), as he found this fraction very heterogeneous in the ultracentrifuge.

I. *Fraction I* is a protein complex containing associated material of acid character primarily, *viz.* nucleic acids, polyphenols and lipids. The amount of lipids and especially that of nucleic acids in fraction I is greatly increased when the extraction is performed at pH 7–8 instead of 5.8. Calcium ion in the concentration of 0.01 M inhibits the extraction of nucleoproteins and partially that of lipoproteins.

The nitrogen content of fraction I varies from 12.5 to 14 % and phosphorus content from zero to 0.6 %. The amount of lipids (determined as ether soluble substance from extracts with alcohol-ether, dried in vacuo) varies from about 3 % in preparations from extracts by 0.01 M calcium sulphate to nearly 20 % from extracts by distilled water adjusted to pH 8.0.

Attempts to estimate the distribution of phosphorus in fraction I according to Schneider² has given the following results:

1) The lipids, extracted with an alcohol-ether mixture, contain only traces of phosphorus. 2) About 20–25 % of the

Fig. 1. Electrophoretic patterns of fractions I and II. Phosphate buffer, $\mu = 0.1$, pH = 7.7. Potential gradients 5.3 volt cm^{-1} (fraction I) respectively 6.9 volt cm^{-1} (fraction II). Exposures after 120 minutes. The numbers indicate ionic mobilities, $10^{-5} \text{ cm}^2 \text{ volt}^{-1} \text{ sec}^{-1}$ as unit.

phosphorus is soluble in cold 5 % trichloroacetic acid (TCA) and thus may derive from phytic acid and inositol esters³.

3) The phosphorus remaining after treatment with cold TCA is released nearly quantitatively by the same reagent at +90° C. Thus 70–80 % of phosphorus in fraction I is probably due to nucleic acids^{2,4}. The presence of nucleic acids is also verified by precipitating the protein moiety by sodium dodecyl sulphate⁵ and measuring the light adsorption of the supernatant between 220 and 300 $\text{m}\mu$. 4) Only traces of phosphorus remain in the precipitate after treatment with heated TCA-solution. The amount of phosphoproteins may thus be regarded as negligible.

The electrophoretic pattern of fraction I is shown in Fig. 1. Despite the great complexity of the fraction the electrophoretic pattern is rather simple and depends very little on the pH-value at the extraction.

Paper electrophoretic experiments have shown that the component with the mobility -6.8 units contains the coloured complexes and lipids. The rapid component with mobility -11.2 units is stained with bromophenol blue like proteins and is found even in phosphorus free preparations.

II. *Fraction II*, precipitated as described above, contains as an impurity some material resembling fraction I. It can be purified by suspending the centrifuged pre-

precipitate with about a 20-fold volume of water, adjusting the pH value first to 7.0 and then to 4.5, centrifuging off the grayish precipitate and reprecipitating the white protein with 2.0 M sodium chloride. Maximum precipitation seems to occur between pH 3.5 and 4.0. Potassium chloride is equally effective as sodium chloride, but ammonium sulphate at the same ionic strength (0.67 M, 16 % saturation) precipitates fraction II only incompletely. Potassium iodide, which has a solubilizing effect on cereal proteins, does not precipitate any protein from this fraction.

Fraction II contains 15.5–16 % N, when prepared from barley and 14.5–15 % N, when prepared from malt. It contains 1.3 % of sulphur, no phosphorus and no or only traces of lipids (at the highest 2 %).

The electrophoretic pattern of fraction II is shown in Fig. 1. The amount of the principal component varies in different preparations from 75 to 96 %. This component seems to be heterogeneous, especially when prepared from barley.

When fraction I is washed or dissolved and reprecipitated, a white precipitate resembling (also electrophoretically) fraction II is obtained from the supernatant by 2.0 M sodium chloride. Thus it may be suggested that fraction I partially consists of complexes, whose protein moiety is similar to fraction II.

Both fractions I and II are resistant to high temperatures and remain soluble in 1 % solutions at +100° C for more than two hours at pH 6.5 ($\mu = 0.01$), but at pH 6.0 fraction II begins to coagulate slowly after 15 minutes. Similar fractions (if not identical) can be precipitated from heated extracts (e. g. from brewery worts).

The amount of these fractions in barley and malt extracts varies depending on the extraction conditions. As much as 31 % of the total nitrogen of barley extract at pH 8.0 is found in fraction I and about 16 % in (crude) fraction II. In extracts obtained at pH 5.8 the corresponding values are 16 and 14 %, respectively, in calcium-containing extracts at pH 7.0, 12 and 11 %. From malt extracts these fractions are obtained in amounts of the same

order of magnitude as from barley extracts, but owing to the greater amount of total nitrogen the relative values are smaller.

The classification of these proteins according to the scheme of Osborne is difficult. Perhaps they might be regarded as globulins, because they are partially precipitated by dialysis. Fraction II, however, is slowly rendered insoluble by dialysis. As heat resistant proteins they are neither albumins, nor can they be glutelin or hordein fractions, because their amide nitrogen content is only 9–10 % of their total nitrogen (determined according to Mecham and Olcott⁶).

The experience in the preparation of these fractions has shown that such common standard methods as precipitation by ammonium sulphate and dialysis are not always ideal. It can be argued that the rather low pH-values used may denature proteins but judging from the solubility of the obtained precipitates the denaturation, if any, is not so great as after ammonium sulphate precipitation.

Further examination is needed to decide, if these fractions contain some earlier characterized barley proteins, e. g. some of the globulins defined by Quensel¹. At present it is not possible to state, whether the nucleoprotein and lipoprotein complexes present in fraction I are naturally occurring in barley grain or if they are artefacts resulting from the extraction.

The author wishes to thank Dr S. Brohult, Mr. H. Nihlén and Dr E. Sandegren for valuable advices and discussions.

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Received October 6, 1955.