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LYSINE-RICH PROTEINS IN HIGH-LYSINE *HORDEUM VULGARE* GRAIN

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Abstract—The salt-soluble proteins in barley grain selected for high-lysine content (Hiproly, CI 7115 and the mutants 29 and 86) and of a control (Carlsberg II) with normal lysine content, contain identical major proteins as determined by MW and electrophoretic mobility. The concentration of a protein group with a high lysine content varies significantly among the barleys examined. One protein, present in large amounts in Hiproly, is assumed to be partially responsible for the high-lysine character of Hiproly, CI 7115 and the mutants 29 and 86.

INTRODUCTION

SINCE Mertz *et al.*[1] found Opaque-2 maize, an intense search for high-protein quality cereals as specified by their nutritional values has been carried out in wheat,[2] rice[3] and barley.[4–6] For most of the cereals, lysine is the limiting essential amino acid and an increased lysine content will lead to an increased nutritional value of the protein. Some high-lysine barleys (HL-barleys) have been found.[4–6]

We presented in a preceding paper[7] a fractionation scheme for the salt-soluble proteins (the proteins soluble in 0·5 M NaCl) of barley revealing differences in the lysine content of the individual proteins. The purposes of the present investigation is to identify lysine rich proteins in the grain of some HL-barleys, and to evaluate the contribution made by such proteins to the overall lysine content of the barley protein complex. Comparisons were also made on the basis of a fractionation of the proteins according to solubility.

The relative content of lysine in the grain protein normally decreases with increasing protein content.[4] The HL-barleys used in the present investigations were selected because of their deviation from this general relationship.[4–6]

RESULTS AND DISCUSSION

The salt-soluble proteins contain from 40% (Hiproly) to 51% (mutant 86) of the total lysine, and the glutelins from 50% (Hiproly) to 32% (mutant 86). These two fractions account for 71% (Hiproly) to 64% (mutant 86) of the protein. Only Hiproly has a lower

[1] MERTZ, E. T., BATES, L. S. and NELSON, O. E. (1964) *Science* **145**, 279.
[2] MATTERN, P. J., SCHMIDT, J. W. and JOHNSON, V. A. (1970) *Cer. Sci. Today* **15**, 409.
[3] TANAKA, S. and TAMARA, S. (1969) *Japan Agr. Res. Quart.* **3**, 1.
[4] HAGBERG, A. and KARLSSON, K. E. (1969) in *New Approaches to Breeding for Improved Plant Protein*, p. 17, IAEA, Vienna.
[5] DOLL, H. (1972) in *Induced Mutations and Plant Improvement*, p. 331, IAEA, Vienna.
[6] VIUF, B. T. (1972) *Kgl. Veterinaer- og Landbohøjskole, Årsskrift*, p. 72.
[7] INGVERSEN, J. and KØIE, B. (1973) *Phytochemistry* **12**, 73.

content of lysine in the salt-soluble protein fraction than in the glutelin fraction. No significant deviations from the control were found in the case of CI 7115 and the Risø mutants 29 and 86. As pointed out earlier[7] it is unlikely that a fractionation according to solubility can elucidate the changes in the individual proteins which lead to the overall increased lysine content. As both lysine content and lysine concentration in the glutelin fraction from Hiproly exceeded the values for the other barleys investigated, it is concluded that at least one lysine rich protein has been increased in the Hiproly glutelin fraction.

Gel Chromatography

Figure 1 shows the protein elution profile of the salt-soluble proteins from the control, mutants 29 and 86, CI 7115 and Hiproly. The peak eluted at V_t,[7] which contains only very small amounts of amino acids and which has a highly variable Folin–Lowry reaction, is not included. The chromatograms in Fig. 1 are transformed to cover 24 mg of protein as determined by the Folin–Lowry method.

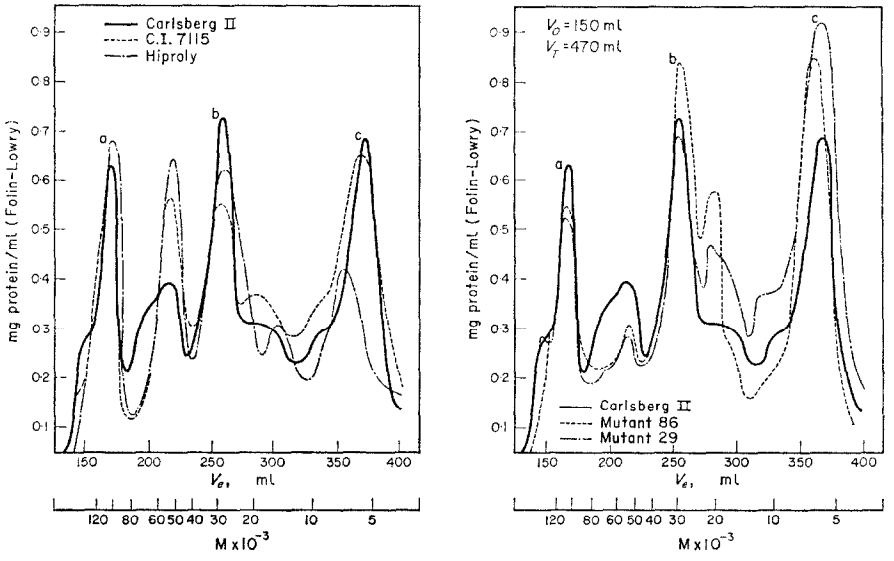

FIG. 1. ELUTION PROFILES OF THE SALT-SOLUBLE BARLEY GRAIN PROTEINS.
The high-lysine barleys were Hiproly, CI 7115, and the mutants 29 and 86, and the normal-lysine control was Carlsberg II. The column (2·5 × 96 cm) of Sephadex G100 (superfine) was equilibrated with 0·05 M phosphate buffer pH 8·0 with 0·45 M NaCl. The effluent was collected in 4·0 ml fractions at the rate of 1·0 ml/cm^2/hr.

We reported[7] that the salt-soluble proteins of Emir barley consists of three major groups when characterized by their apparent MWs. In all the five barleys represented in Fig. 1, the same three peaks (the a-, b- and c-peaks) are found, together with other peaks, which are present to a variable extent in the different barleys. Between the a- and b-peaks, a humped peak in the control apparently represents two proteins, one of which is present in large amounts in CI 7115 and Hiproly. Just after the b-peak a protein seems to be lacking completely in Hiproly but is relatively abundant in the mutants 29 and 86. The c-peak of Hiproly is eluted a little earlier than are the other c-peaks. Only minor differences exist between the elution profiles of the two mutants.

Figure 2 illustrates the elution profiles of the salt-soluble lysine from mutant 29, Hiproly and CI 7115. Each profile is transformed to cover 10 mg of lysine. No significant differences could be detected among the profiles of the control line (Carlsberg II) and mutant 29. The lysine content of the a-peak is fairly equal for the three HL-barleys. The major part of the salt-soluble lysine from Hiproly is found in the b-peak, which contains a much smaller part of the lysine in CI 7115 and mutant 29. Based on three chromatograms, 46–50% of the salt-soluble lysine from Hiproly is present below the b-peak (from V_e 240 to V_e 300 ml), compared to from 33 to 36% for the control, mutant 29 and CI 7115. Besides the lysine contribution from the minor peak with V_e 325 ml (Fig. 2), only very small amounts of lysine are found in the low MW fractions of the Hiproly elution profile. However, the low MW c-peak contributes substantially to the overall lysine content of the salt-soluble proteins of the control and mutant 29. Figure 2 also shows that CI 7115 has a high content of lysine, which originates from low MW proteins, and a concomitant lower content of lysine from the b-peak, compared with all other barleys investigated.

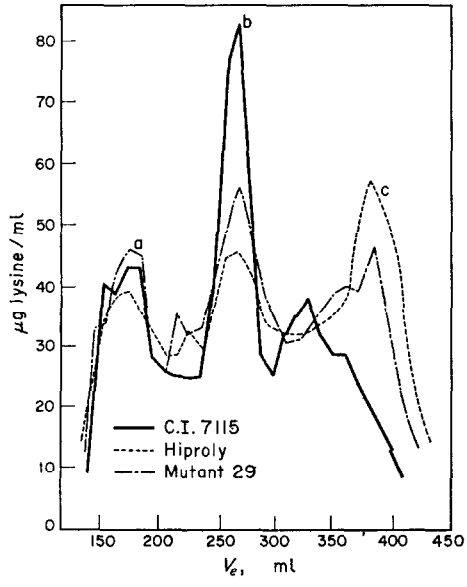

FIG. 2. SEPHADEX G100 SUPERFINE ELUTION PROFILE OF THE SALT-SOLUBLE PROTEIN LYSINE FROM THE HIGH-LYSINE BARLEYS HIPROLY, CI 7115 AND MUTANT 29.

Of the major proteins, only the b-peak proteins from the HL-barleys deviate by elevated lysine content. The maximum lysine concentration of the b-proteins are 7·6, 8·5 and 8·4% for mutant 29, Hiproly and CI 7115, respectively. The lysine concentrations of the proteins from the control was 7·3% lysine for V_e 295 ml. Though only small differences exist in the overall lysine contents of the salt-soluble proteins between the control and the mutants 29 and 86, the lysine distribution among the individual salt-soluble proteins exhibits some characteristic differences, which may be connected with the HL-character of the mutant. Besides the similarities among the HL-barleys concerning the b-peak proteins, the mutant proteins have a maximum lysine concentration of 8·0% for V_e 350 ml. In this region the Hiproly proteins, too, contain much lysine. Hiproly deviates from the other barleys by

having a pronounced variation in the lysine content of the salt soluble proteins (3·4–8·6%). The major b-protein, which contributes 46–50% of the salt soluble protein from Hiproly, contains 8·6% lysine. In the case of CI 7115, only the lysine content of the b-peak proteins, which reach the values of those from Hiproly, deviates significantly from that of the control. As shown earlier,[7] the b-peak is composed of a protein group containing four major proteins. The varying lysine contents of the b-peak proteins are possibly caused by changed relative representation of the individual proteins.

Electrophoresis of the Salt-soluble Proteins

The protein composition of the collected fractions was determined by disc-electrophoresis. If an individual protein is defined as a discrete band arriving at a certain elution volume, almost the same patterns are obtained for the salt-soluble protein of the five barleys investigated, and for the previously investigated Emir barley.[7] Thus the high-lysine b-peak includes identical proteins for the five barleys examined.

The supposed high-lysine protein from Hiproly[8] is identified as one of four b-peaks proteins. Except in the case of Hiproly, there are no essential genotypic differences in the amounts of the four proteins as determined by the colour intensity of the protein bands. Hiproly is especially rich in one of the b-peak proteins. It is, therefore, assumed to be a high-lysine protein.

V_e for the c-peak maximum of Hiproly is 355 ml compared with 375 ml for the mutants and CI 7115. This is owing to the presence of a protein which is strongly represented in the salt-soluble fraction of Hiproly but not, or only weakly, in the remaining HL-barleys. This protein may be the high-lysine protein suggested in the V_e 325 ml-region (Fig. 2), but in the mutants it may not be sufficiently represented to express itself as a protein band. Outside the regions which contain proteins with high lysine content the salt-soluble fraction of Hiproly contains two proteins with V_e 220 ml, which cannot be found in the other electropherograms. A protein in the V_e 220 ml-region must exist in the other samples, too, since the elution patterns (Fig. 1) show protein peaks at this position, but this protein is not found with the disc-electrophoretic system used. Two proteins with V_e 205 ml and 240 ml are not present in Hiproly. The latter is presumably responsible for the pronounced valley found in Hiproly elution pattern just after the b-peak (Fig. 1).

The facts that the b-peak includes identical proteins in all five barleys examined and that the relative amounts of those proteins have changed significantly in the case of Hiproly suggest that one of the reasons for the lysine superiority of Hiproly is owing to a change in the control mechanism which regulates the synthesis of the quantitatively important b-proteins. To a minor extent such a change has possibly also taken place in the case of the mutants and CI 7115.

EXPERIMENTAL

Material. The HL-barley material was grown in two groups. Group 1, which was grown in the field, included the following HL-barley mutants: Risø 29 and 86.[5] Group 2 was grown in pots in the open air and included two HL-barley varieties from the world collection: Hiproly[4] and CI 7115 (Ref. 6, variety No. 468). Carlsberg II was grown as a control in both groups.

Nitrogen, protein and lysine determinations. Nitrogen was determined colorimetrically after conversion into ammonia, the indophenol method[9] being used. Protein was calculated as N × 6·25. The gel chromatography protein elution profile was determined by the Folin–Lowry method[10] on 4·0 ml fractions. Only

[8] INGVERSEN, J. and KØIE, B. (1971) *Hereditas* **69**, 319.
[9] ROMMERT, P. J. and VISSER, J. (1969) *Analyst* **99**, 653.
[10] LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J. (1951) *J. Biol. Chem.* **193**, 265.

nitrogen eluted when $V_e < V_t$ is considered to be due to protein. Lysine was determined by hydrolyzing 40 mg finely ground barley with 2 ml 6 N HCl for 18 hr at 100° in 15 ml Pyrex vials with Teflon lined screw-caps. In the case of the protein eluate, 1 ml was hydrolyzed with 1 ml 12 N HCl, the same procedure being used. The analysis was carried out on a Beckman 120C amino acid analyzer by the method prescribed by the manufacturer.

Protein fractionation after solubility, gel chromatography, disc-electrophoresis and the preparation of the salt-soluble protein fraction were carried out as described earlier.[7]

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