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LYSINE RICH PROTEINS IN THE SALT-SOLUBLE PROTEIN FRACTION OF BARLEY

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Key Word Index—*Hordeum vulgare*; Gramineae; barley; protein; quality; albumin; globulin; lysine; fractionation.

Abstract—Fractionation of the protein complex from Emir barley showed that the salt-soluble fraction accounts for 44% of the total lysine content but only for 24% of the total nitrogen. If glutelin is included, these two fractions covered 82% and 57% respectively. A highly reproducible method that is non-denaturing and recovers approximately 75% of the salt-soluble nitrogen is described. Twenty-two per cent of the extracted salt-soluble nitrogen consisted of low molecular weight nitrogen compounds, which only contribute 7% of the fractions lysine content. The lysine content of the individual salt-soluble proteins varied between 5·3 and 7·3%. The existence of three high-lysine protein groups in the salt-soluble protein fraction of barley is suggested. The significance of these findings in relation to the present work on quality improvement of barley protein is discussed.

INTRODUCTION

IN RECENT years cereals superior in lysine have been found.[1–4] From a nutritional point of view, these findings are important because lysine is the first limiting essential amino acid of all important cereals. The changed overall lysine content has been associated with an altered seed morphology, and also the protein composition as specified by solubility criteria (Osborne fractionation)[5] has been changed in maize,[1] and in barley.[2,6] The lysine rich cereal lines had a decreased relative content of the lysine poor prolamine fraction.[1,2,6]

The object of our investigation is to localize lysine-rich proteins in the protein complex of barley varieties with normal- and high-lysine contents, and to explain the changes found in the overall lysine content. The present study is of the lysine balance in a normal trade barley variety 'Emir', based on the lysine content of the individual Osborne fractions. The study also includes the protein composition of and the lysine distribution in the salt-soluble nitrogen fraction.

The distinction between albumin and globulin is based on solubility criteria that are difficult to reproduce and which are unimportant in the present investigation. The salt-soluble nitrogen fraction is defined as the nitrogen extracted with 0·5 M NaCl. Numerous studies have been devoted to the evaluation of the heterogeneity of the barley proteins in

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this fraction (for review see Refs. 7–11) and genotype specific protein patterns have been demonstrated.[12,13] We have earlier demonstrated the genotypic differences in electrophoretic protein patterns between a normal trade variety and the high-lysine barley, Hiproly.[14]

RESULTS and DISCUSSION

Protein Fractionation

Table 1 gives the lysine and protein ($N \times 6{\cdot}25$) distributions on the individual Osborne fractions for Emir barley containing 12·6% protein. The protein and lysine recovery was 98% and 87%, respectively. The low lysine recovery is possibly due to the different conditions during the hydrolysis of the individual fractions. In particular, the high carbohydrate content of the salt-soluble fraction may cause a loss of lysine. Forty-four per cent of the total lysine content is present in the salt-soluble fraction, which contains 24% only of the total protein ($N \times 6{\cdot}25$). The salt-soluble fraction account, together with the glutelins, for 82% of the total lysine content, but only for 57% of the total protein content. The hordein (barley prolamine) fraction comprises only 9% of the overall lysine content but 35% of the protein content.

TABLE 1. LYSINE AND PROTEIN CONTENTS OF EMIR BARLEY

Fraction	Total	Salt-soluble proteins	Hordein	Glutelin	Insoluble proteins
Protein g*/100 g grain	12·6	3·01	4·45	4·19	0·64
% of total protein	—	24	35	33	5·1
Lysine mg/100 g grain	381	145	30	126	31
% of total recovered lysine	—	44	9·0	38	9·3
% Lysine in protein	3·03	4·82	0·67	3·01	4·84

Recovery of protein: 12·29 g ~ 98%; recovery of lysine: 332 mg ~ 87%. * $N \times 6{\cdot}25$.

After 5 successive standard extractions (see Experimental), 18% of the total grain nitrogen was extracted. This corresponds to 75% of the salt-soluble fraction as it is determined by the modified Osborne method (Tables 1 and 2). The low extraction temperature of the standard method is the main reason for the decreased extraction yield. The first extract contains $92 \pm 2\%$ of the total amount of nitrogen which can be extracted by successive extractions according to the standard procedure (Table 2). The protein pattern of this first extract determined by disc electrophoresis (pH 9·5) is identical with the pattern of the protein in the succeeding extracts. In order to overcome the problem of concentrating big volumes, only one extraction is carried out. This extract is considered representative of 75% of the

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salt-soluble nitrogen corresponding to 33% of the total lysine content. Table 3 shows how the nitrogen content of the standard extract (fraction 2), and the nitrogen and lysine content of the concentrate derived from the extract (fraction 3), are related to the total nitrogen and lysine contents of the grain. The very small amount of diffusible nitrogen and lysine—10% of the extracted nitrogen and 3% of the extracted lysine—is not accounted for in the following.

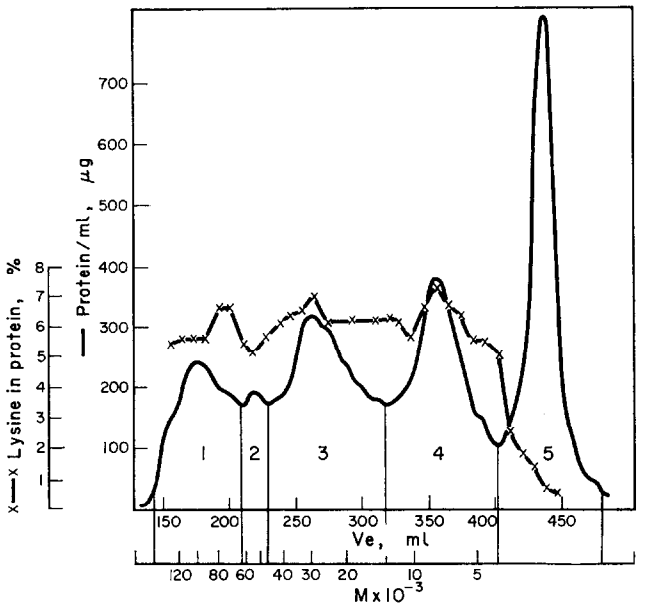

FIG. 1. SEPHADEX G100 SUPERFINE ELUTION PROFILE OF THE SALT-SOLUBLE NITROGEN FRACTION FROM EMIR BARLEY.
The column (2·5 × 88 cm) was equilibrated with a 0·05 M phosphate buffer 8·0 with 0·45 M NaCl. The effluent was collected in 4·50 ml fractions at the rate of 1·10 ml/cm²/hr. The lysine content in the collected proteins is calculated on the basis of nitrogen and lysine determinations on 9·0 ml fractions.

Gel Chromatography of the Salt-soluble Nitrogen Fraction

Figure 1 shows 4 major peaks, (1, 3, 4 and 5), around which the major part of the salt-soluble nitrogen of Emir barley is gathered. The 5-peak (22% of the salt-soluble nitrogen) is positioned at V_T, and consequently consists of low molecular weight compounds. From 73 to 97% of the eluted nitrogen is recovered as amino acid nitrogen (Table 5) in fractions with V_E < 400 ml, while only 23% of the 5-peak-nitrogen is amino acid nitrogen. The major part of this peak is thus made up of unidentified low MW nitrogen compounds. The positive Folin reaction of the compounds in the 5-peak may be attributed to the presence of various phenolic amines which are known to exist in some barley tissues.[15] It is of particular interest here that the lysine content of the salt-soluble proteins becomes twice as high as the overall lysine content of the barley seed proteins, when the non-protein nitrogen of the 5-peak is excluded. Thus 12% of the barley protein contributes 28% of the barley

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lysine. It is evident that the regulatory mechanism behind the synthesis of these particular proteins must be of special importance in the breeding work for high lysine barleys.

TABLE 2. YIELD OF PROTEIN ($N \times 6{\cdot}25$) BY SUCCESSIVE EXTRACTIONS OF 10·0 g DEFATTED FLOUR FROM EMIR BARLEY WITH 100 ml SOLVENT ACCORDING TO THE STANDARD PROCEDURE

Extract No.	1	2	3	4	5
% of total protein extracted	16·2	1·38	0·37	0·06	0·0
% of albumin + globulin extracted	90*	7·6	2·1	0·4	0·0
mg protein extracted	204	17·3	4·7	0·8	0·0

* Based on 4 repetitions, this figure is 92·3 ± 1·8%.

The pooled fractions 1, 3, 4 and 5 (Fig. 1, Table 4) constitute 18–26% of the salt-soluble nitrogen fraction of Emir barley, while the protein is distributed with 23, 33 and 33% of the overall salt-soluble protein in the fractions 1, 3 and 4 respectively. The uneven distribution of lysine among the salt-soluble barley proteins appears from the last column and is clearly demonstrated when lysine is determined in each of the collected fractions (Fig. 1). Lysine-rich proteins are located especially around the 70, 28 and 7·6 M positions, i.e. at least one high lysine (HL) protein is positioned under each of the three major protein peaks of Fig. 1 with minimum lysine contents of 6·6, 7·0 and 7·3% respectively. The presence of shoulders and the asymmetric peak shape of particularly the 1- and 3-peaks indicate the presence of several proteins below these peaks, while the shape of the 4-peak suggests a more simple protein pattern. This is confirmed by disc electrophoresis on the collected fractions. Concerning the high lysine regions, no proteins have concentration maximum in the 70 M position. Four and two bands occur at the 28 and 7·6 M positions respectively. The question whether all these bands represent HL-proteins has not been further investigated.

TABLE 3. LYSINE AND NITROGEN CONTENTS OF THE VARIOUS SALT-SOLUBLE NITROGEN FRACTIONS

	Protein fraction	Nitrogen content mg/10 g grain	Nitrogen content % of salt-soluble nitrogen	Nitrogen content % of total nitrogen	Lysine content mg/10 g grain	Lysine content % of salt-soluble lysine	Lysine content % of total lysine	% lysine in protein ($N \times 6{\cdot}25$)
1	Salt-soluble fraction (see Table 1)	48·9	100	24	14·5	100	38	4·8
2	Standard extract	34·0 ± 1·5*	70	17	—	—	—	5·2
3	Standard concentrate (non diffusible mat.)	28·2 ± 0·4*	63	15	10·6 ± 0·2	73	28	5·6
4	Diffusate	3·3	7	1·8	0·4	3	0·1	1·8
5	Rest (1 − 2)	14·9	30	7·5	3·5	24	9·2	3·9

* s.e. are based on 4 repetitions.

The various protein fractions have been analyzed for total amino acid contents (Table 5). Again the 5-peak segregates by having a very high content of, especially, Asp and to a smaller extent, also of Glu and Ala, covering together 56% of the total amino acid content of this peak. The 4-peak proteins are especially characterized by high content of Asp, Ser and especially Cys (9%), and low contents of Glu, Ala and Phe, giving an indication of a less heterogeneous protein composition of the peak.

It is concluded that the salt-soluble proteins of Emir barley include at least three major proteins possibly belonging to the reserve proteins, with apparent MWs around 70,

28 and 7·6 M, and with lysine contents exceeding that of the whole fraction by 9, 16 and 21% respectively. Raising the lysine content of barley is a question of modifying the genetic control mechanism responsible for the regulation of the reserve protein synthesis.[16]

TABLE 4. NITROGEN, PROTEIN AND LYSINE CONTENTS OF THE SALT-SOLUBLE NITROGEN COMPOUNDS OF EMIR BARLEY SEPARATED ON SEPHADEX G100 SUPERFINE

Fraction No.	mg	Nitrogen content % of total recovered nitrogen	mg	Protein content % of total recovered protein	μg	Lysine content % of total recovered lysine	% lysine in protein
1	2·85	18·3	17·8	23·4	1035	21·0	5·83
2	1·25	8·0	7·8	10·3	428	8·7	5·47
3	4·05	26·0	25·3	33·3	1550	31·4	6·13
4	4·02	25·8	25·1	33·0	1591	32·2	6·34
5	3·39	21·8	—	—	334	6·8	—
Total	15·56	99·9	76·0	100·0	4938	100·1	6·06

Recovery of nitrogen: 99%; recovery of lysine: 95%.

Therefore, besides the obvious interest in increasing the content of salt-soluble protein, genotypic differences in the content of especially the HL-proteins referred to above is of particular interest in the breeding work for improving the barley protein quality. Genotypic differences of the above-mentioned type are being searched for in HL-barleys and will be reported on later.

TABLE 5. AMINO ACID COMPOSITION OF THE MAJOR SALT-SOLUBLE NITROGEN FRACTIONS OF EMIR BARLEY (mole %)

Elution volume (ml)	180	217	252	352	436	Elution volume (ml)	180	217	252	352	436
Lys	7·6	8·0	8·6	9·1	3·4	Cys	1·2	2·0	5·2	9·1	4·2
His	3·7	3·4	2·2	2·8	2·1	Val	7·4	7·0	7·8	6·5	2·9
Arg	10·8	7·2	8·6	11·4	4·2	Met	1·8	2·6	3·2	1·6	0·8
Asp	10·7	11·2	13·8	14·9	23·7	Ile	4·2	4·2	2·8	4·5	3·1
Tre	5·4	6·8	6·2	5·3	2·9	Leu	8·4	8·7	8·8	8·0	3·6
Ser	6·3	5·6	5·9	7·5	4·3	Tyr	4·2	5·1	4·5	4·2	0·9
Glu	18·7	17·8	13·7	9·9	19·0	Phe	6·0	5·2	4·2	1·8	2·0
Pro	5·2	6·5	6·4	7·1	3·7						
Gly	7·1	6·9	6·7	8·1	12·7	Recovered nitrogen					
Ala	7·9	8·6	8·2	5·4	6·6	(%)	83	73	82	97	23

EXPERIMENTAL

Material. Hordeum vulgare (cv. Emir) was grown in the field at Risø in 1970 and harvested at maturity. The seeds contained 9% H_2O after defatting (see Osborne fractionation).

Nitrogen, protein, and amino acid determinations. Nitrogen was determined colorimetrically after conversion into ammonia, the indophenol method[17] being used. Protein was calculated as $N \times 6{\cdot}25$. The gel chromatographical protein elution profile was determined by the Folin–Lowry method[18] on 4·5 ml fractions and by nitrogen determinations on 9·0 ml fractions. Only nitrogen eluted when $V_E < V_T$ is considered as belonging to protein. Amino acid determinations were carried out by ion exchange chromatography.[19] Hydrolysis was made in vacuum at 110° for 18 hr. No corrections are made for loss during hydrolysis. Samples for lysine determination were hydrolysed in Pyrex vials with Teflon lined screw caps.

Modified Osborne fractionation. The seeds were ground in a hammer mill and defatted with cold acetone (10 ml/g) by stirring 18 hr at −21°. After drying by washing with Et_2O, 2·00 g were extracted at room temp. with 20 ml solvent in a 20-mm dia. round bottomed centrifuge tube by vigorous stirring with a 15-mm Teflon coated magnet. Three different solvents were used (albumin, globulin and non-protein nitrogen: 0·5 M NaCl, 0·05 mM EDTA–Na; hordein: 55% v/v *iso*-propanol; glutelin: 0·0125 M $Na_2B_4O_7$, 0·043 M

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NaOH, 0·5% sodium dodecylsulphate) and each solvent step was repeated twice (albumin, globulin and non-protein nitrogen: 3 × 30 min; hordein and glutelin: 2 × 30 min + 1 × 18 hr). The extracts were clarified by centrifugation (25 000 *g*, 30 min, 20°) and pooled for nitrogen and lysine determinations.

Preparation of the salt-soluble protein fraction—the standard procedure. All procedures were performed in N_2 at 4° and all solutions were preflushed with N_2. *Extraction.* 10·0 g of defatted flour was extracted by agitation for 1 hr at 4° with 100 ml 0·5 M NaCl being 5 mM Cys-HCl, 2·5 mM ascorbic acid and 2·7 mM EDTA–Na. The extract was clarified by centrifugation (8000 *g*, 30 min, 4°). *Concentration.* The extract (*ca.* 90 ml) was concentrated by dialysis against 20 g polyethyleneglycoll (M = 20 000). Approximately 20 ml concentrate was collected after 16 hr.

Gel chromatography. 10·00 ml of the standard concentrate was applied to a 2·5 × 88 cm Sephadex G100 superfine column and eluted with phosphate buffer (I = 0·05, pH 8 with 0·45 M NaCl and 7·7 mM NaN_3) at a rate of 1·11 ml/cm²/hr. Calibration was made with bovine serum albumin 114 M, 67 M ovalbumin 45 M, trypsin 24 M, and cytochrome-*c* 14 M (M stands for the factor 10^3).

Disc electrophoresis. 7·5% polacrylamide was used at two pH-stages—9·5 and 2·3—on 100 μl of the eluted fractions.[20,21]

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