Purification and Structural Characterization of LTP1 Polypeptides from Beer

Sandrine Jégou,[†,‡] Jean-Paul Douliez,*[,†] Daniel Mollé,[§] Patrick Boivin,[‡] and Didier Marion[†]

Unité de Biochimie et Technologie des Protéines, INRA, BP 71627, 44316 Nantes Cedex 03, France, and Institut Français des Boissons et de la Brasserie Malterie, Pôle Technologique de Brabois, 7 rue du Bois-de-la-Champelle, BP 267, 54512 Vandoeuvre Cedex, France, and Unité de Recherches de Technologie Laitière, INRA, 35042 Rennes Cedex, France

We report on the purification of lipid transfer proteins (LTP) from barley seeds and beer with the aim of investigating the chemical modifications that occur during the brewing process. In seeds, the well-known LTP of 9 kDa (LTP1) has been found together with a second form named LTPb that displays comparable amino acid composition but was not fully sequenced. These two forms have been recovered in beer with marked chemical modifications including disulfide bond reduction and rearrangement and especially glycation by Maillard reaction. The glycation is heterogeneous with variable amounts of hexose units bound to LTPs. Circular dichroism shows that glycated LTP1 having all their disulfide bridges reduced are totally unfolded. These results provide a first basis for understanding how barley LTPs become foam-promoting agents during the malting and brewing process.

Keywords: *Barley; beer; lipid transfer protein; glycation; foam*

INTRODUCTION

In brewing technology, the formation, stability, and texture of foams are important aspects of beer quality. The foam properties are closely related to the beer constituents such as alcohol, carbohydrates, melanoidins, metal ions, iso-α-acids, and proteins (Bamforth, 1985). The protein material includes mainly the 9-kDa lipid transfer protein (LTP1) and protein Z, two barley albumins that survive the malting and brewing processes. Both LTP1 and protein Z are heat stable and are not or are weakly degraded by proteolytic enzymes during malting and brewing. In a first attempt, this can be related to the amino acid sequence homologies of these proteins with those of amylase and protease inhibitors. Thus, protein Z is structurally related to serpins, members of the serine protease inhibitor family (Hejgaard et al., 1985; Brandt et al., 1990). Although barley LTP1 was first placed in the amylase/protease inhibitor family (Svensson et al., 1986; Bernhard and Sommerville, 1989), a role in the inhibition of green malt cysteine endoproteinases was suggested (Jones and Marinac, 1997), but that hypothesis has been refuted (Davy et al., 1999).

Protein Z has been the first characterized protein in beer where it contributes to foam stability and/or haze formation (Hejgaard, 1977; Kaersgaard and Hejgaard, 1979; Yokoi et al., 1989). Protein Z with a molecular mass of about 43 kDa is composed of different isoforms with p*I* in the range of 4.5−5.5 (Yokoi et al., 1989). It has been estimated that about 16% of the lysine content of protein Z are glycated during the brewing process through Maillard reaction (Hejgaard and Kaersgaard, 1983).

LTP1 is an abundant soluble protein of the aleurone layers from barley endosperm (Mundy et al., 1986). It is characterized by a p*I* of about 9 and consists of 91 amino acid residues for a molecular mass of 9694 Da (Svensson et al., 1986). The compact structure of the barley LTP1 comprises four helices stabilized by four disulfide bonds and a well-defined C-terminal arm with no regular secondary structure (Heinemann et al., 1996). In comparison to other plant LTP1 (Tassin et al., 1998; Charvolin et al., 1999), the barley protein has a small hydrophobic cavity (Heinemann et al., 1996) but is capable of binding different lipids such as fatty acids and acyl-CoA (Lerche et al., 1997; Lerche and Poulsen, 1998). It must be noted that a 7-kDa isoform of these lipid transfer proteins has also been evidenced in barley (Kalla et al., 1994) and is referred to as LTP2.

LTP1s are surface-active proteins that adsorb at air−water interfaces (Subirade et al., 1995; Subirade et al., 1996) but display poor foaming properties (Sørensen et al., 1993). It has been shown that barley LTP1 is modified during the brewing process, leading to foam-promoting forms that concentrates in beer foams (Bech et al., 1995). These LTP1 forms contribute to foam formation, while foam stability depends on protein Z (Sørensen et al., 1993). However, these modifications of barley LTP1 that occur during the brewing process have not been fully investigated, and the reasons why LTP1 then possesses foaming properties are unknown. Therefore, the aim of this paper is to describe, for the first time, the structural and chemical modifications that occur on barley LTP1 during the malting and brewing processes, by comparing the proteins isolated from seeds and beer.

* To whom correspondence should be sent [phone: (33) (0) 240 67 50 56; fax: (33) (0) 240 67 50 25; e-mail: douliez@nantes.inra.fr].

[†] Unité de Biochimie et Technologie des Protéines, INRA.

[‡] Institut Français des Boissons et de la Brasserie Malterie, Pôle Technologique de Brabois.

[§] Unité de Recherches de Technologie Laitière, INRA.

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MATERIALS AND METHODS

Extraction and Purification of Barley and Beer LTP1. Soluble proteins were extracted from 1.5 kg of whole barley flour (*Hordeum vulgare*, cv. Plaisant) by gentle stirring with 4 L of distilled water for 4 h at room temperature. After being centrifuged, the soluble material was lyophilized for purification. For beer LTP1 isolation, 20 L of lager-type beer brewed at an industrial scale (Heineken) and at a pilot scale (IFBM) were degassed and lyophilized.

The lyophilized barley extract was solubilized in 3 L of MES (2*N*-morpholino-ethansulfonic acid) 20 mM, pH 5.6, buffer and loaded on a cation exchange Streamline SP (Pharmacia, France) column (5 × 30 cm). To remove low molecular weight compounds, the lyophilized beer material was first dialyzed (dialysis tubing cutoff 3.5 kDa) against deionized water and then against MES buffer before loading on the cation exchange column. Proteins were eluted at a flow rate of 30 mL/min with a linear gradient from 0 to 700 mM NaCl in MES buffer. The collected fractions were analyzed by SDS−PAGE in the presence of 2-mercaptoethanol. The fractions containing proteins with molecular mass around 10 kDa were pooled, dialyzed against deionized water, and freeze-dried. The dry material was solubilized in MES buffer (1 g/10 mL) and loaded on a Sephadex G-50 (Pharmacia, France) column (100 × 2.7 cm) equilibrated with MES buffer. Elution was carried out at a flow rate of 50 mL/h, and the eluted fractions were analyzed by SDS−PAGE. The fractions containing proteins below 10 kDa were fractionated by semipreparative reversed phase HPLC (RP-HPLC) using a column (25 × 1 cm) packed with C18 bonded silica Nucleosyl, 5 μm, 300 Å (CIL, Bordeaux, France). Elution was performed at 50 °C with a gradient of water−acetonitrile−0.04% TFA from 20 to 50% acetonitrile for 30 min at a flow rate of 3 mL/min, and proteins were detected by absorbance at 280 nm. The collected fractions were lyophilized after dilution in deionized water.

Mass Spectrometry. Protein molecular masses were measured using a Perkin-Elmer API III$^+$ (Sciex, Thornill, Canada) triple quadrupole mass spectrometer equipped with an atmospheric pressure ionization source (Electro-spray mass spectrometer, ES-MS) (Fenn et al., 1989). The sample analysis (1 mg/mL) was achieved by an online coupling between MS and RP-HPLC (LC-MS). Elution was carried out on a RP-HPLC column (Symmetry C18 Waters, Milford, MA) at a flow rate of 0.25 mL/min (40 °C) with a split to the MS ionization source that was set at a flow rate of 30 μL/min. Ion detection was performed in positive mode, and molecular masses were determined from charge *m*/*z* using Biomultiview 1.2 (Software package Sciex).

Amino Acid Analysis. Amino acid compositions were determined by the Bidlingmeyer method (Bidlingmeyer et al., 1984) with a Picotag analyzer (Waters, Milford, MA) after hydrolysis in 6 M HCl for 24 h at 110 °C.

Amino Acid Sequencing. N-terminal amino acid sequencing was performed by Edman degradation on a model 477A gas-phase sequencer. The phenylthiohydantoin amino acids were analyzed online by RP-HPLC using a 120A analyzer (Applied Biosystems, Foster City, CA).

Reduction and Alkylation. A total of 1 mg of protein was solubilized in 500 μL of Tris-HCl 0.5 M, pH 8.5, containing guanidine-HCl 6 M and EDTA 0.01 M. Reduction was performed by adding 0.1 M dithiothreitol (DTT). After 2 h at 50 °C under nitrogen, alkylation was carried out with iodoacetic acid in 0.5 M NaOH. The reduced and alkylated proteins were fractionated by RP-HPLC on a C18 Nucleosyl 5 μm, 300 Å bonded silica column (25 × 0.46 cm) as described for semipreparative RP-HPLC except that the flow rate was 1 mL/min and detection was performed at 220 nm.

Circular Dichroism Spectroscopy. The secondary structure of proteins was determined by circular dichroism (CD) in the far UV (from 190 to 250 nm). The measurements were performed at 25 °C on a CD6 Jobin-Yvon dichrograph. Proteins were solubilized in deionized water at a final concentration of 0.5 mg/mL. A quartz cell of 0.2-mm path length was used. Data were expressed as mean residue ellipticity. Secondary structures were determined by the method of Provencher and Glöckner (1981). A mean residue mass value of 106.4 was used.

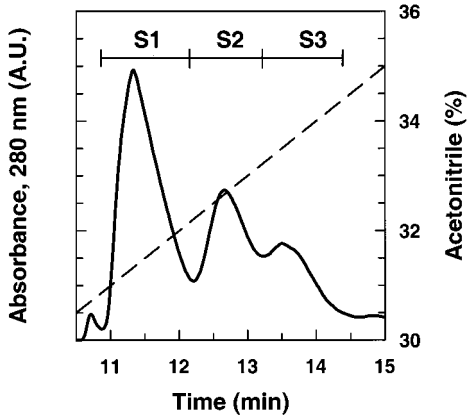

Figure 1. Semipreparative reverse-phase HPLC pattern of the LTP1 purified from barley seeds after cation exchange and size exclusion chromatographies. S1, S2, and S3 represent the fractions that were recovered for further analysis.

Analysis for Free Sulfhydryl Groups in Protein Solutions. Colorimetric reactions were conducted under the conditions described by Ellman (1958). A total of 2 mg of protein was suspended in 3 mL of buffer containing 6 M urea and 3 mM EDTA, pH 8. One hundred microliters of DTNB [5,5-dithiobis(2-nitrobenzoic acid)] solution (40 mg of DTNB solubilized in 10 mL of buffer) was added into the solution. The reaction mixture was incubated at room temperature for 15 min. The absorbance was recorded at 420 nm against a protein-free blank containing the DTNB assay solution.

Carbohydrate Analysis. Carbohydrate contents were determined by the phenol/sulfuric method (Dubois et al., 1956). A total of 1 mg of protein was dissolved in 200 μL of water. A 5% phenol solution and 1 mL of concentrated sulfuric acid were added with the protein. Absorbance was measured at 490 nm, and glucose content was determined using a reference curve.

Carbohydrates were also analyzed by gas-phase chromatography. A total of 5.2 mg of protein were hydrolyzed for 90 min at 120 °C in the presence of 0.5 mL of 4 N TFA and 0.5 mL of inositol as internal standard (1 mg/mL). Sugars were reduced by $NaBH_4$ for 1 h at 40 °C and were acetylated by 2 mL acetic anhydride/0.2 mL of *N*-methylimidazole for 20 min at room temperature. The mixture was washed with water and chloroform. The chloroform phase was analyzed on a capillary column DB225 (30 m × 0.32 mm) at 200 °C and 0.6 bar.

RESULTS

Characterization of Purified Barley and Beer LTP1s. *Purification of LTP1 and Protein Molecular Mass Determination.* The RP-HPLC pattern of barley LTP1 recovered after cation exchange and size exclusion chromatographies showed three fractions S1, S2, and S3 that are eluted around 30% acetonitrile (Figure 1). Sequencing of the first five amino acids returned a sequence strictly analogous to that of LTP1. Electrospray mass spectrometry revealed a major compound for fraction S1 with a molecular mass of 9689 Da (Figure 2a), which was in agreement with the theorical mass of LTP1 (Svensson et al., 1986). A minor peak of mass 9525 Da was also detected (Figure 2a) and corresponded to LTP1 having lost the C-terminal tyrosine residue. This is known to occur with extraction of proteins in the case of barley because of the presence of numerous carboxypeptidases in seeds (Mikola, 1983). A main peak with molecular mass of 9983 Da was obtained for fractions S2 and S3 (Figure 2b). Then the two fractions were pooled, and the protein was named LTP1b. As for LTP1,

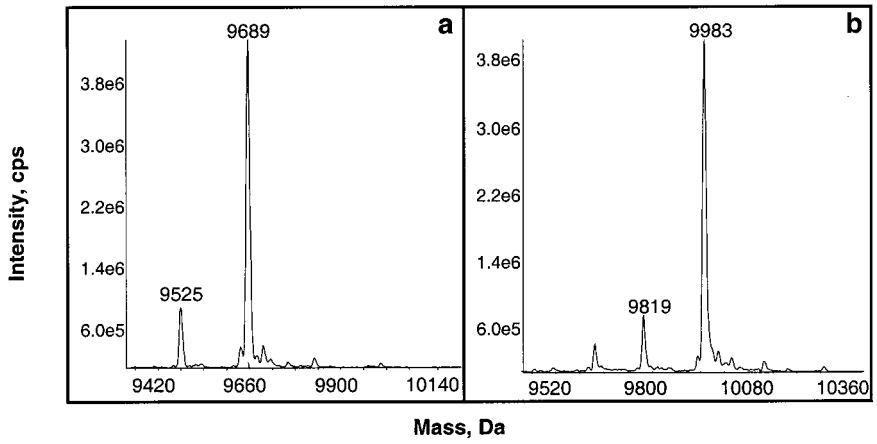

Figure 2. Deconvoluted and reconstructed electrospray mass spectra from multicharged ions spectra of purified LTP1 (a) and LTP1b (b) from barley seeds.

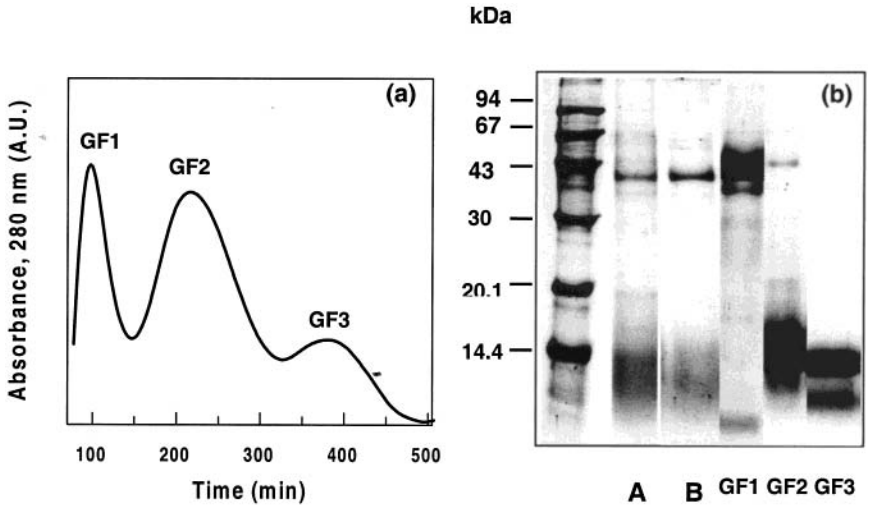

Figure 3. (a) Chromatographic pattern of beer proteins on Sephadex G-50 size exclusion column; (b) SDS−PAGE of the proteins of original beer (lane A), the proteins eluted by cation exchange chromatography (lane B), and the proteins eluted on Sephadex G-50 in fractions GF1, GF2, and GF3.

Table 1. Amino Acid Composition of LTP1 and LTP1b Purified from Barley Seeds (Plaisant)

	LTP1 mol %	LTP1b mol %
Asx	16.1	15.4
Thr	3.4	2.6
Ser	8.3	7.0
Glx	8.7	7.8
Pro	8.8	8.3
Gly	11.0	10.2
Ala	4.7	4.2
Val	6.6	6.4
Met	1.0	1.5
Ile	5.8	6.6
Leu	6.8	6.7
Tyr	1.3	1.6
His	1.5	1.7
Lys	3.5	3.7
Arg	3.5	3.5
Cys	nd[a]	nd

[a] nd, not determined.

a mass corresponding to the LTP1b having lost its C-terminal tyrosine residue was observed by mass spectrometry (Figure 2b). Fluorescence experiments revealed that LTP1b enhanced intermembrane lipid exchange (not shown) as LTP1. Furthermore, identical N-terminal amino acid sequences were identified for LTP1 and LTP1b, and slight differences were observed for the amino acid composition of the two proteins (Table 1). However, no further analysis was done to determine the full sequence of LTP1b.

The beer material was submitted to the same purification procedure. From degassed beer, SDS−PAGE revealed the presence of two major bands (Figure 3b, lane A). In a first attempt, the band with an apparent molecular mass around 40 kDa was attributed to protein Z, while the smeared band with apparent molecular mass around 10 kDa was attributed to LTP1. Cation exchange chromatography of this beer material revealed a broad peak (not shown) where both protein Z and LTP1 were recovered (Figure 3b, lane B). This protein Z/LTP1 fraction was dialyzed overnight and lyophilized before size exclusion chromatography. Three fractions, named GF1, GF2, and GF3 were obtained (Figure 3a). SDS−PAGE (Figure 3b) and amino acid analysis (results not shown) suggested that protein Z was the main protein of fraction GF1. SDS−PAGE revealed another sharp band with a molecular mass around 4−5 kDa (Figure 3b, lane GF1), which was not further identified. Fraction GF2 showed a broad band of apparent molecular masses in the 8−12 kDa range (Figure 3b, lane GF2). Fraction GF3 revealed two bands of apparent molecular masses around 7 and 10 kDa (Figure 3b, lane GF3). GF1 was discarded from the

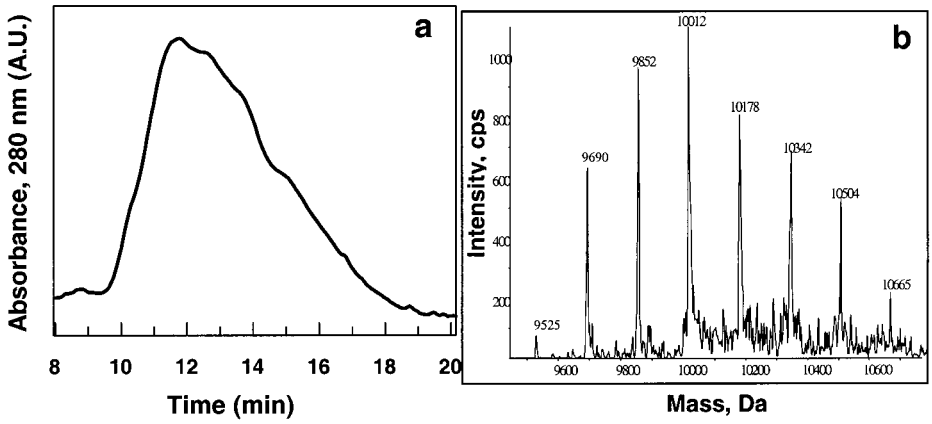

Figure 4. (a) Semipreparative RP-HPLC pattern of the fraction GF2 isolated by size exclusion chromatography and collected as fraction B1. (b) Deconvoluted and reconstructed electrospray mass spectrum from multicharged ions spectrum of fraction B1 from beer.

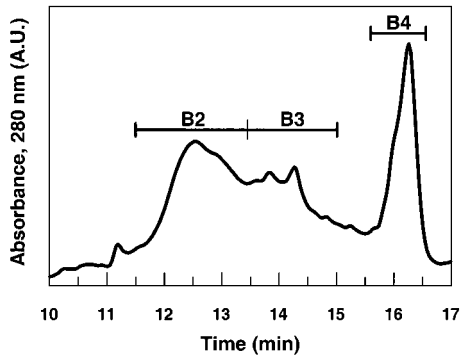

Figure 5. Reverse-phase HPLC of the fraction GF3 after size exclusion chromatography.

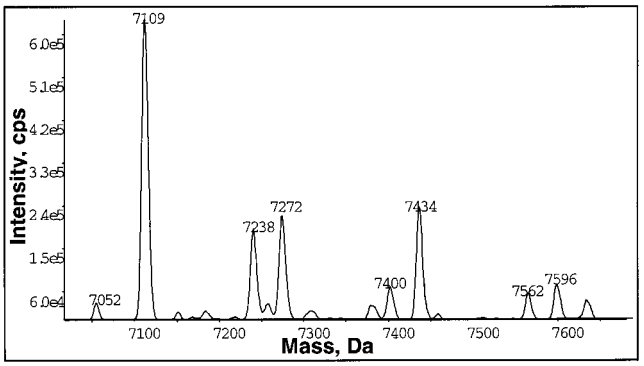

Figure 6. Deconvoluted and reconstructed electrospray mass spectrum from multicharged ions spectrum of the purified protein of the fraction B4 from beer.

purification, and both GF2 and GF3 fractions were submitted to RP-HPLC for further analysis.

For fraction GF2, RP-HPLC provided a broad single peak eluted at about 30% acetonitrile and was referred as fraction B1 (Figure 4a). The mass spectrum of fraction B1 from beer showed multiple peaks with molecular masses in the range of 9526−10 504 Da (Figure 4b). Both masses of 9690 and 9525 Da corresponded to native LTP1 and to the protein with the C-terminal tyrosine removed, as found in barley seeds, respectively. Other masses corresponded to adducts of about 162 ± 2 Da, meaning that one or several molecules were covalently bound to LTP1. Considering the mass of adducts, these were supposed to be hexoses as previously observed for dairy proteins (Nacka et al., 1998). The carbohydrate content was confirmed by the phenol/sulfuric method, and a GPC carbohydrates assay showed that B1 contained about 5% of sugars as adducts corresponding to glucose units that were covalently grafted on the proteins.

The RP-HPLC pattern of fraction GF3 displayed a wide peak eluted from 31.5 to 35% of acetonitrile that was collected in two fractions B2 and B3 and was followed by a sharp peak B4 around 36% of acetonitrile (Figure 5). The mass spectra of fractions B2 and B3 also showed numerous masses in the range of 9526−11 260 and 9820−10 984 Da, respectively (not shown). As compared to B1, it appeared that fraction B2 corresponded to an analogous fraction except that the range of mass was broader, suggesting in this case that more adducts of 162 Da were bound to the proteins. Mass spectrometry analysis (not shown) revealed that B3 corresponded to LTP1b with numerous adducts of 162 Da. For fractions B1, B2, and B3, the proteins were identified as being LTP1 or LTP1b by sequencing of the four N-terminal amino acids, and then they were named B1/LTP1, B2/LTP1, and B3/LTP1b. It was also shown in these cases that proteins contained about 5% of sugars as adducts.

Unexpectedly, the mass spectrum of fraction B4 showed a major product with a molecular mass of 7109 Da and minor proteins with molecular masses in the range of 7052−7596 Da, i.e., differing by the number of the 162-Da adduct (Figure 6). The amino acid sequencing of the four N-terminal amino acids highlighted two sequences, Leu−Asn−Cys−Gly and Ala−Ala−Cys−Glu, which are found in the primary structure of barley LTP1 (Svensson et al., 1986) and LTP2 (Kalla et al., 1994). After reduction and alkylation on fraction B4 of disulfide bonds, RP-HPLC revealed three major peaks (Figure 7), which were collected and submitted to sequencing of the first four amino acid residues. Peptide 1 corresponded to the native unreduced fraction B4, while peptide 2 provided the amino acid sequence, Leu−Asn−Cys−Gly and peptide 3, Ala−Ala−Cys−Glu. These data suggested that fraction B4 were composed of two polypeptides connected by at least a disulfide bond and derived from partial hydrolysis of the 7-kDa (LTP2) and the 9-kDa (LTP1) lipid transfer proteins. However, no further analysis was performed to obtain more information on these peptides.

Finally, by weighing the freeze-dried material, the yield of the different protein fractions could be estimated (Table 2). Thus, quite similar amounts of LTP1 and

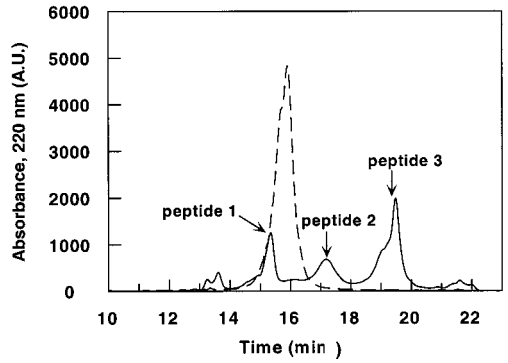

Figure 7. Analytical reverse phase HPLC of fraction B4 before (—) and after (- - -) reduction and alkylation.

Table 2. Lipid Transfer Protein Purification from Barley Seeds (Plaisant) and from Beer, Secondary Structure of LTP1, and Estimation of Free Sulfhydryl Groups[a]

	quantity of purified protein	secondary structure	free −SH
barley flour	(mg/kg)		
LTP1	103.3	α-helix	nd[b]
LTP1b	82.7	α-helix	nd
lager beer	(mg/L)		
B1/LTP1	19.9	unordered	8
B2/LTP1	11.9	α-helix	4
B3/LTP1b	4.6	α-helix	2
B4 fraction	8.4	nd	

[a] The amount of total protein was obtained by weighing the powder recovered after lyophylization. The secondary structure was estimated by the method of Provencher and Glokner, while the free S-H content was determined by titration with DTNB (see text). [b] nd, not determined.

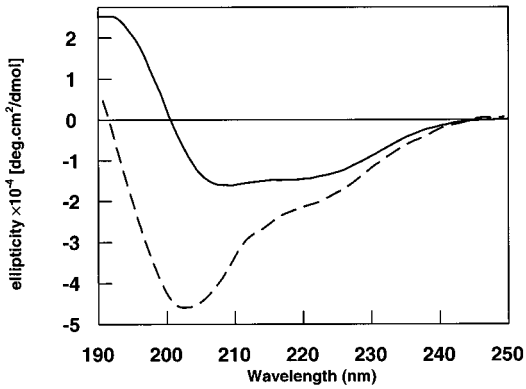

Figure 8. CD spectra of the LTP1 from barley (—) and the B1/LTP1 (- - -) from beer at concentration 0.5 mg/mL in ultrapure water.

LTP1b were isolated from barley seeds, while glycated LTP1 was the major isoform recovered from beer.

Determination of the Secondary Structure and Free Sulfhydryl Groups in LTP1 Solution. The CD spectrum (Figure 8) of LTP1 purified from barley seeds revealed a double minimum at 222 and 208 nm, which was characteristic of an α-helix structure. In contrast, the CD spectrum of the B1/LTP1 from beer displayed a strong negative band around 202 nm with a shoulder between 220 and 230 nm, suggesting that the protein was denatured. In this case, the percentage of α-helix estimated by the method of Provencher and Glöckner (1981) was about 12%. The α-helix, β-sheet, and random coil contents of other beer and barley LTP1s were also estimated. The results showed similar α-helix contents, around 50%, for barley LTP1 and LTP1b as well as for beer fractions B2/LTP1 and B3/LTP1b. The secondary structure of each LTP1 forms isolated from barley seeds or beer was reported in Table 2. The α-helix structure determined for the LTP1 is in agreement with structure displayed by Heinemann et al. (1996). In the case of beer forms, the results revealed that B3/LTP1b and a part of LTP1 have kept their secondary structure throughout the brewing process.

Because it was supposed from CD measurements that beer proteins could be denaturated, it appeared interesting to determine the amount of free cysteine residues. These results obtained by titration of free thiol groups with DTNB were displayed in Table 2. As expected by CD experiments, the B1/LTP1 from beer exhibited its eight cysteine as free thiol groups, while B2/LTP1 and B3/LTP1 revealed four and two reduced disulfide bridges, respectively.

DISCUSSION

Unexpectedly, two related LTP1 isoforms, LTP1 and LTP1b, with molecular masses of 9689 and 9983 Da, respectively, have been isolated from barley seeds. Furthermore, the proportion of these isoforms are almost identical. LTP1 corresponds to the previously described LTP1 from barley aleurone layers (Svensson et al., 1986; Skriver et al., 1992), while a protein with a mass corresponding to that of LTP1b has been reported recently (Evans and Hejgaard, 1999). Because of the identity of their N-terminal amino acid sequences, presence of C-terminal tyrosine residue, and close amino acid composition, it was first suggested that LTP1 and LTP1b could be isoforms exhibiting only a few differences in their amino acid sequences. This is in agreement with the fact that lipid transfer proteins form a multigenic family (Kader, 1996). In this regard, it is noteworthy that close amino acid sequences are displayed by some LTP1 isoforms from barley leaves (Molina et al., 1993). However, Skriver et al. (1992) showed that barley LTP1 was encoded by a single gene, and the protein accumulated specifically in the aleurone layer of developing and germinating seeds. Although a carbohydrate assay reveals that LTP1 and LTP1b are not glycosylated, the possibility of another posttranslational modification cannot be excluded.

Previous experiments that have reported the purification of LTP1 from beer foam reveals different components with molecular masses in the range of 9600−9900 Da (Bech et al., 1995), and a mass of 9975 Da was specifically identified (Lusk et al., 1995). These data suggest that LTP1b has been already observed in beer but never further characterized. The absence of this isoform in previous works cannot be due to a genetic variability since LTP1b has also been isolated from Scarlett and Esterel, spring and winter barley cultivars (results not shown). Furthermore, LTP1b was recovered from a commercial lager-type beer that is obtained from a mixture of different barley malts. This result can be related to the difference in purification the procedure that has been used in previous studies. Here, the same procedure has been used to purify LTP1 from barley and beer, while in the previous works, barley LTP1 has been isolated by a procedure involving ammonium sulfate precipitation steps (Svensson et al.,1986; Sørensen et al., 1993). Therefore, it can be suggested that LTP1b is removed or altered during these precipitation steps.

Purification of beer proteins shows that the two main proteins, LTP1 and protein Z, cannot be well separated by cation exchange chromatography, which could sug-

gest interactions between the two proteins during this step. However, the second step of purification, size exclusion chromatography on Sephadex G-50 gel, permitted the separation of protein Z and LTP1. LTP1s recovered from beer after purification showed that these proteins are apparently weakly or not degraded by the proteases during the malting and brewing processes. Only the C-terminal tyrosine residue can be removed, but this is also found to occur in seeds. The resistance of LTP1 to the numerous enzymes that are present along the brewing process can be explained by the glycation of the protein. This is emphasized by the fact that protein Z, which is also glycated, survives the process. Indeed, the presence of adducts with a molecular mass of 162 Da on these LTP1 forms and the detection of glucose suggest that LTP1 is glycated by Maillard reactions. Maillard reaction is a well-known nonenzymatic reaction that can form covalent linkages between reducing sugars and protein-free amino groups (Maillard, 1912). The reaction is favored by high-temperature treatments during the brewing process (Hejgaard and Kaersgaard, 1983). The most reactive groups are the α-NH_2-terminal amino acids and the ϵ-NH_2 of the side chain of lysine residues, which lead by condensation with sugars to the formation of stable ketoamines or Amadori compounds (Hodge, 1955). The four lysine residues of LTP1 are the potential sites of glycation. Since similar glycation patterns are found for LTP1 and LTP1b it is probable that they are composed of the same number of lysine residues. The masses obtained indicate that glycation of the LTP1 is heterogeneous. This can be due to the presence of both glucose and maltose in barley malt (Allosio et al., 2000).

It has been shown that the unfolding of LTP1 occurs during wort boiling of the brewing process (Bech et al., 1995). Concerning the structure of LTP1, we observed modifications of B1/LTP1 with an important unfolding of the polypeptide chain and cleavage of all the disulfide bonds. The other glycated forms maintain an α-helix structure although some of their disulfide bonds are cleaved. This suggests that the unfolding can occur only when all the disulfide bonds are reduced. Such an unfolding has been already observed with wheat LTP1 (Désormeaux et al., 1992). On the other hand, carbohydrates grafted on protein might protect the polypeptide fraction from precipitation during mashing, boiling, and fermentation (Pierce, 1978).

Concerning the minor fraction B4, the two N-terminal sequences determined corresponded to the 7 kDa LTP2 (Kalla et al., 1994) and the 9 kDa LTP1 of barley. It is suggested that this fraction is formed by two peptides connected by disulfide bridges and that are derived from the two lipid transfer protein named before by proteolysis of the C-terminal region during the brewing process. This form shows that some proteolysis of LTP1 can occur during the malting and/or brewing processes. Interestingly, the presence of this dimer suggests that a specific interaction should occur between LTP1 and LTP2. This interaction should take place early in the process, probably on extraction of malt with water to allow the disulfide bond rearrangement on brewing. Reduction and rearrangements of disulfide bonds occur under the redox conditions found during the malting and brewing processes (Boivin et al., 1999).

Regarding foam formation, glycation, disulfide bond reduction, and unfolding provides a first explanation for the better efficiency of LTP1 from beer to form foam than native barley LTP1 (Sørensen et al., 1993). Hydrophobic groups and amphipathic domains of unfolded LTP1 should easily adsorb at air−water interfaces of beer foam. Furthermore, the unfolding of the other forms at air−water interfaces can be favored by the partial disulfide bond cleavage. In this regard, it is noteworthy that the foaming properties of whey proteins are correlated with an increase of unfolding ability favored by disulfide bond cleavage (Kella et al., 1989). Glycation should reinforce the surface properties of these forms by increasing their amphiphilicity and solubility. Furthermore, hydrophilic carbohydrates are localized in the liquid between the bubbles (Roberts, 1975). This leads to an increase in viscosity, which can reduce the liquid drainage from foam (Bamforth, 1985). However, it has been suggested that proteins contribute to beer foam across ionic bonds between negative charges of isohumulones and ϵ-amino group of lysine residues to form more surface-active complexes (Asano and Hashimoto, 1976). Such interactions are probably impaired by the decrease of positive charge of LTP1 by the covalent linkage of carbohydrates to the amine group of lysine side chains.

Therefore, for the first time, the presence of two closely related LTP1 isoforms, named LTP1 and LTP1b, has been highlighted in barley seeds. LTP1 corresponds to previously described LTP1, while LTP1b can be a new isoform or a posttranslationally modified LTP1. Further analysis has to be done on this new isoform that is present in most barley cultivars. These proteins are weakly degraded by proteolytic enzymes throughout the brewing process. However, these LTP1 isoforms undergo different modifications such as disulfide bond reduction and rearrangements, hydrolysis, and glycation by the Maillard reaction. This provides a first basis for understanding the foam-promoting effect of barley LTP1 in beer. The surface and foaming properties of the different beer LTP1 forms have to be characterized. Finally, purification of LTP1 along the brewing process will allow the identification of the key stages of the brewing process where these modifications occur.

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