Immunological Study of Hydrophobic Polypeptides in Beer

E. N. Clare Mills,*[,†] Juliet A. Kauffman,[†] Michael R. A. Morgan,[†] J. Michael Field,[‡] Jørn Hejgaard,[§] Michael O. Proudlove,[∥] and Akiko Onishi[∥]

Department of Biochemistry, Institute of Food Research Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom; Advanta Seeds U.K., Docking PE31 8LS, United Kingdom; Department of Biochemistry and Nutrition, Building 224, Technical University of Denmark, DK-2800 Lyngby, Denmark; and Brewing Research International, Lyttel Hall, Nutfield, Surrey RH1 4HY, United Kingdom

A panel of 15 monoclonal antibodies (mAbs) has been raised to a fraction of lager beer protein precipitated with ammonium sulfate at 40% saturation. The antibodies have been characterized in terms of their binding to a set of Octyl-Sepharose fractions of lager beer foam with increasing hydrophobicity (groups 1−5) by enzyme-linked immunosorbent assay and immunoblotting methods. The epitopes recognized were present in all of the lager beer foam fractions to some degree, and many of the mAbs bound to both high molecular mass (MM) (>3000 Da) and low MM (<3000 Da) material. Two antibodies, IFRN 1612 and 1613, bound primarily to the high MM material and preferentially recognized the more hydrophobic foam groups important in beer foam formation and stability. All of the mAbs recognized proteins present in malt, with much of the binding directed toward the material soluble in aqueous propanol from the highly modified crystal malt used in the lager beer production. Such a library of mAbs will be of use in investigating the contribution made by proteins and derived polypeptides, such as those from malt, to quality attributes such as foaming.

Keywords: *Beer; foam; malt; monoclonal antibodies; immunoassay*

INTRODUCTION

One particular characteristic of beer that influences consumer perceptions as to product quality is the formation and appearance of a stable head of foam. Proteins and polypeptides make an important contribution to foam quality, although the role they play is not clearly defined (Bamforth, 1985). The structure of foam is complex, with a network of hexagonal bubbles**,** the walls of which comprise surface active components, including proteins and polypeptides. Interactions between these components that give rise to desirable foaming quality are poorly understood. Fractionation of beer components on the basis of size and hydrophobicity has been used to identify key surface active polypeptides (Bamforth, 1985). In general, fractions of higher molecular mass polypeptides (i.e., >5000 Da) have the greatest foaming potential (Anderson and Harris, 1963; Asano and Hashimoto, 1980), as do those of greater hydrophobicity (Slack and Bamforth, 1983; Onishi and Proudlove, 1994).

The protein content of beer has been estimated to be ∼0.5 g L^{-1} (Kaersgaard and Hejgaard, 1979), although the figures obtained depend on the method of analysis and may in some instances also include nonprotein nitrogen (Bamforth, 1985). Beer proteins and polypeptides range in molecular mass from <1000 Da (Dale and Young, 1989) to >90000 Da (Asano and Hashimoto, 1980), and many appear to be present as aggregates in beer (Sørensen and Ottesen, 1978; Dale and Young, 1992). Most beer proteins and polypeptides have been shown to originate from the malt by virtue of (1) being rich in the amino acids glutamine and proline, a characteristic of the barley storage proteins known as hordeins (Dale et al., 1989), and (2) immunological studies indicating the presence of barley-related proteins (Grabar and Daussant, 1971; Hejgaard and Sørensen, 1975; Kauffman et al., 1994; Sheehan and Skerritt, 1997). It has also been demonstrated that yeast proteins are present in beer, albeit as minor constituents (Hejgaard and Sørensen, 1975; Mohan et al., 1992). As the heterogeneity of barley proteins is further increased by modification during malting and brewing, it is not suprising that few beer proteins have been purified to homogeneity. The best characterized is protein Z (Hejgaard, 1977), an M_r 40000 protein found in barley, which remains virtually unchanged throughout the malting and brewing process and which has been estimated to make up ∼20−170 mg/L of beer protein. Another purified protein, associated with foam formation, is a barley lipid transfer protein 1 (LTP 1; Sørensen et al., 1993; Bech et al., 1995), which has an M_r of ∼9700, estimated by gel electrophoresis.

In addition to classical biochemical methods of analysis such as chromatography and electrophoresis, researchers have also employed immunochemical techniques to study beer proteins. Polyclonal antisera against barley, malt, beer, and yeast proteins have been employed to investigate the origin of beer proteins, to remove polypeptides from beers to establish their effect on foaming (Hollemans and Tonies, 1989), to analyze beer for foam and haze proteins (Ishibashi et al., 1996; Mohan et al., 1992), and to measure the content of a

* Author to whom correspondence should be addressed (telephone 44-1603-255295; fax 44-1603-507723; e-mail Clare.Mills@bbsrc.ac.uk).

† Institute of Food Research Norwich Laboratory.

‡ Advanta Seeds.

§ Technical University of Denmark.

∥ Brewing Research International.

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40000 Da foam protein (probably protein Z) in beer and its relationship with foam stability (Horiuchi et al., 1996). However, in general, the information that can be obtained using polyclonal antisera is limited partly because of the problem of polyspecificity, which results from the presence of immunodominant repetitive sequences from hordeins in beer (Mills et al., 1995); in some instances, polyclonal antibodies have to be affinity purified to remove anti-hordein antibodies (Bech et al., 1995). Such problems are circumvented by using monoclonal antibodies (mAbs) targeting a single epitope. They have been employed to examine modification of hordeins (Skerritt, 1988; Skerritt and Henry, 1988) and limit dextrinase (Sissons et al., 1992) during malting and also to characterize hordein-derived beer and foam polypeptides (Kauffman et al., 1994; Sheehan and Skerrit, 1997). Food proteins that undergo thermal and proteolytic processing, such as the storage proteins of soybean (Plumb et al., 1994, 1995) and pea (Quillian et al., 1990), have also been investigated using mAbs, but to date there are no reports of mAbs being raised to beer proteins. The present paper describes the production and characterization of a panel of mAbs developed to lager beer proteins; the data obtained show that many of the immunologically reactive beer polypeptides originate in malt.

MATERIALS AND METHODS

Materials. Bicinchoninic acid (BCA) protein assay kit was from Pierce and Warriner, Chester, U.K. Freund's adjuvant was from Difco (Detroit, MI). Nunc Immunoplate I microtitration plates were from Gibco BRL Life Technologies Ltd. (Paisley, U.K.). Ultrafiltration membrane (YM3 DIAFLO with molecular weight cutoff of 3000 Da) was from Amicon Ltd. (Stonehouse, U.K.). Peroxidase and alkaline phosphatase labeled anti-rabbit IgG, plus anti-mouse IgG/IgM, avidin−horseradish peroxidase conjugate, nitroblue tetrazolium, 5-bromo-4-chloro-3-indolyl phosphate (BCIP), Nonidet NP-40, and bovine serum albumin (BSA) were from Sigma Chemical Co., Poole, U.K. Substrate based on 3,3′,5,5′-tetramethylbenzidine (TMB) was from Vetoquinol, Bicester, U.K. Kathon was from Rohm and Haas (Croydon, U.K.). Nitrocellulose membrane was from Sartorius Ltd., Epsom, U.K., Octyl-Sepharose CL-4B was from Pharmacia Biotech (Uppsala, Sweden), and prestained molecular weight markers were from Bio-Rad (Hemel Hempsted, U.K.). All other reagents were of AR grade.

Barley and Malt Samples. Lager beer for immunogen preparation and foam fractions, together with the pilsner and crystal malts used in its production, was obtained from a commercial brewery. Malting barley cultivars (cv.) Blenheim and Puffin were supplied by Advanta Seeds U.K. (Docking, U.K.), whereas the malted barley cv. Puffin was produced using pilot-scale maltings at BRI (Nutfield, U.K.).

Lager Beer and Malt Fractions. A protein fraction of a commercial lager beer was prepared by precipitation at 4 °C with $(NH_4)_2SO_4$ at 40% saturation (termed the ammonium sulfate fraction), followed by dialysis of the resulting precipitate using a 10000 Da cutoff membrane, against water prior to freeze-drying. A stock solution of this fraction was prepared in 4% (v/v) ethanol for use as the immunogen and stored at −20 °C. Foam fractions of differing hydrophobicity (groups 1−5) were prepared from the same lager beer by hydrophobic interaction chromatography on Octyl-Sepharose CL-4B as described by Onishi and Proudlove (1994) and fractionated further into high and low MM fractions by ultrafiltration through a 3000 Da cutoff membrane.

The majority (pilsner-type) and minority (crystal-type) malts were ground, and 1 g was extracted with shaking in 2 × 10 volumes (i.e., 10 mL) of 1 M NaCl for 1 h at 25 °C. After centrifuging at 10000*g* for 30 min at 25 °C, the supernatants (containing the salt-soluble malt components and termed the salt fraction) were decanted and pooled. The resulting pellets were rinsed with water and re-extracted with 2 × 10 volumes (i.e., 10 mL) of 50% (v/v) propan-1-ol, 2% (v/v) acetic acid, and 2% (v/v) 2-mercaptoethanol by shaking for 1 h at 25 °C. Extracts (essentially containing residual prolamins and termed the propanol fraction) were centrifuged as described above and the supernatants retained and pooled. Salt- and propanol-soluble materials were then dialyzed against water at 4 °C, freeze-dried, and stored at −20 °C until required. A salt-soluble fraction of barley (cv. Blenheim) was produced in the same way. The protein contents of extracts were determined using a BCA assay kit.

Antibody Preparations. Polyclonal antisera were raised in rabbits to purified protein Z and β-amylase as described by Hejgaard (1982). mAbs were produced essentially as described by Mills et al. (1990) with the following modifications. Mice were immunized with the ammonium sulfate fraction of lager beer, which was also used for mAb selection. All cell culture procedures were performed using OptiMEM (Gibco, U.K. Ltd.) supplemented with 4% foetal calf serum (FCS; from Advanced Protein Products Ltd., U.K.) as the culture medium. mAbs were isotyped using an isotyping kit (Sigma Chemical Co.), and culture supernatants were concentrated as required using Centriprep centrifugal concentrators (30000 Da cutoff membrane, Amicon).

ELISA Procedures. For selection of mAbs, Nunc Immunoplate I microtitration plates were coated (inner 60 wells only) with 0.3 mL/well of the immunogen (i.e., the ammonium sulfate fraction of lager beer at 1 μg/mL) in 0.05 M sodium carbonate−bicarbonate buffer, pH 9.6. After overnight incubation at 4 °C, plates were washed three times with water (using a Wellwash 5000, Life Sciences International U.K. Ltd., Billingshurst, U.K.), allowed to dry in air, and stored at −20 °C until required. For cross-reactions with lager beer fractions, plates were coated using a Biomek 1000 (Beckman Instruments, High Wycombe, U.K.) with a different fraction in each quarter, the lower right quarter always being coated with the 40% ammonium sulfate fraction of lager beer.

For screening of hybridomas, culture medium (0.15 mL/well) was transferred from culture to assay plates and incubated overnight at 4 °C. Plates were then washed five times in phosphate-buffered saline containing Tween 20 [PBST; 0.14 M NaCl, 0.0015 M KH_2PO_4, 0.008 M Na_2HPO_4, 0.0027 M KCl; pH 7.4, containing 0.05% (v/v) Tween 20] prior to the addition of 0.2 mL/well of anti-mouse IgG horseradish peroxidase conjugate diluted 1:1000 (v:v) in PBST. Following a 3 h incubation at 37 °C, plates were washed a further five times in PBST before the addition of 0.2 mL/well of substrate based on 3,3′,5,5′-tetramethylbenzidine (TMB). After the color had been allowed to develop for 10 min at 37 °C, the reaction was stopped by the addition of 50 μL/well of 2 M H_2SO_4, and the absorbance values of the wells were determined at 450 nm using a Titertek MCC plate reader (Flow Laboratories, Thame, U.K.). For cross-reactions, identical titration curves were set up on each coated quarter plate using the Biomek 1000, with either culture supernatant diluted appropriately in PBST, or rabbit anti-protein Z or β-amylase antisera; plates were then developed as described above using anti-rabbit IgG labeled with horseradish peroxidase for those plates set up with the rabbit antisera. In this way comparable titration curves could be obtained for all of the fractions of interest. From these the cross-reactivities (as a percentage) were calculated as the ratio of binding observed to each lager beer fraction to the binding observed to the 40% ammonium sulfate fraction of lager beer, at a particular antibody dilution. For the polyclonal antisera cross-reactions were calculated relative to a salt-extract of barley (cv. Blenheim).

Electrophoretic and Immunoblotting Procedures. Grain (barley or malt) was crushed and sonicated in 3.33% (w/v) SDS, 0.067 M Tris-HCl, pH 6.8, 10% (v/v) glycerol, and 0.001% (w/v) Pyronin Y containing 100 mM DTT and heated to 100 °C for 3 min. Beer and foam fractions were brought to the same buffer composition by the addition of 50% (v/v) glycerol and concentrated sample buffer [0.67 M Tris-HCl, pH 6.8, containing 33.3% (w/v) SDS and 0.01% (w/v) Pyronin Y

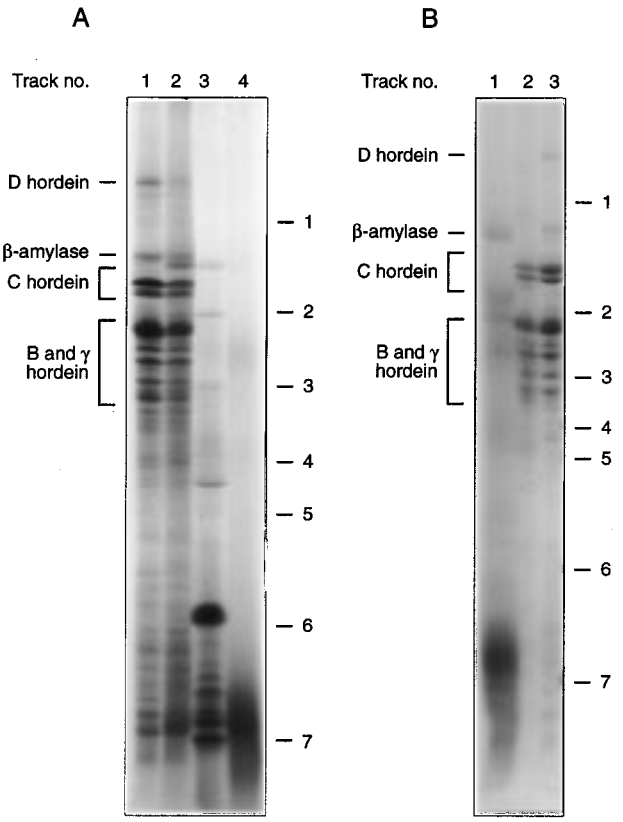


Figure 1. SDS−PAGE analysis of barley, malt, and lager beer fractions: (A) track 1, total barley seed proteins cv. Puffin; track 2, total malt proteins cv. Puffin; track 3, 40% ammonium sulfate fraction of lager beer; track 4, lager beer foam group 5; (B) track 1, pilsner malt salt fraction; track 2, pilsner malt propanol fraction; track 3, barley from which pilsner malt was derived. Parts A and B show gels run at different times. Molecular weight markers were as follows for both gels: 1, bovine serum albumin, 66000; 2, hen egg ovalbumin, 45000; 3, rabbit muscle glyceraldehyde-3-phosphate dehydrogenase; 4, bovine erythrocyte carbonic anhydrase, 29000; 5, bovine pancreatic trypsinogen, 24000; 6, soybean trypsin inhibitor 20000; 7, bovine α-lactalbumin, 14000.

containing 1 M DTT] and boiled as above. Barley, malt, and beer fractions were analyzed using a Tris−borate buffer system as described by Koenig et al. (1970) with a 15% acrylamide−0.1% bis(acrylamide) separating gel and a 3% acrylamide−0.08% bis(acrylamide) stacking gel using a vertical gel electrophoresis system (Hoefer Scientific, U.K.).

Foam fractions were separated using a 15% polyacrylamide gel Laemmli system (Onishi and Proudlove, 1994) with prestained molecular weight markers. Immunoblotting was performed essentially as described by Mills et al. (1990) using a 0.2 μm pore nitrocellulose membrane and an alkaline phosphatase/BCIP−nitroblue tetrazolium detection method.

RESULTS

A panel of 15 mAbs was developed to an ammonium sulfate fraction of lager beer, the composition of which was determined by SDS−PAGE (Figure 1A, track 3). The major components of this fraction were an $M_r \sim$ 20000 polypeptide and two low molecular mass polypeptides of <10000 M_r. Neither was visible in the samples of barley, malt, or foam group 5 of lager beer analyzed (Figure 1A, tracks 1, 2, and 4 respectively). The reactivities of the mAbs were determined by ELISA against a set of Octyl-Sepharose fractions of lager beer foam, the hydrophobicity and foaming potential of which increased from group 1 through group 5 (Onishi and Proudlove, 1994). The effect of polypeptide size on mAb reactivity was also determined, as the lager beer foam groups had been further fractionated into high MM and low MM fractions using ultrafiltration through a 3000 Da cutoff membrane. Finally the origin in malt of the lager beer polypeptides was determined using the mAb probes.

Reactivity of mAbs with Lager Beer Polypeptides. In general, mAb epitopes were present to various degrees in foam fractions (cf. Figure 2A−F), with hydrophilic foam group 1 polypeptides being the least well recognized of the foam fractions. On the basis of their cross-reactivities, the mAbs could be categorized in terms of the foam group they reacted with most strongly (Figure 2), as follows: group 2, low MM, IFRN 1604, 1608, 1619 (Figure 2A); group 3, high MM, IFRN 1605 (Figure 2B); group 4, high MM, IFRN 1611 (Figure 2C); group 4, low MM, IFRN 1615, 1618, 1622, 1623 (Figure 2D); group 5, high MM, IFRN 1612, 1613, 1620 (Figure 2E); group 5, low MM: IFRN 1621 (Figure 2F).

Greater reactivity was observed toward the more hydrophobic groups, with over half the mAbs binding most strongly to foam groups 3−5. Apart from the mAbs that strongly recognized group 5 high MM (Figure 2E), all of the antibodies showed considerable reactivity with the low MM fractions recognizing epitopes contained within polypeptide fragments of <3000 Da (Figure 2A−D,F). Indeed, certain antibodies predominantly recognized low MM polypeptides of foam groups 2 (IFRN 1604, 1608, and 1619), 4 (IFRN 1615, 1618, 1622, 1623), and 5 (IFRN 1621), respectively, with a lesser reaction toward the high and low MM fractions of the other foam groups. Some antibodies primarily recognized the high MM material of hydrophobic foam groups 3 (IFRN 1605) and 5 (IFRN 1612, 1613, and 1620).

SDS−PAGE analysis showed all foam groups to have very similar compositions, with a major polypeptide of M_r (40000 and a range of poorly resolved polypeptides of M_r 6000−20000 being evident (Figure 3A). Immunoblots developed with the library of mAbs gave several types of reactivity patterns, and representative examples are shown in Figure 3. Two mAbs, IFRN 1612 and 1613 (parts B and C of Figure 3, respectively), while both predominantly binding to foam group 5 high MM polypeptides by ELISA, gave quite different reaction patterns on immunoblotting. The former mAb bound only weakly to immunoblots, recognizing primarily an $M_r \sim$ 50000 polypeptide that was present in all foam groups. Some binding to lower molecular mass material ($M_r \sim$ 30000−35000) was evident as a smear on the immunoblot in groups 1 and 2 (Figure 3B, tracks 1 and 2). In contrast, IFRN 1613 bound strongly to a low M_r species of ∼8000 Da present in all foam groups and a smaller polypeptide that ran just behind the dye front, which was only present in foam groups 1 and 5. A trace of binding to the $M_r \sim$ 50000 polypeptide, also recognized by IFRN 1612, was observed on the immunoblot for all foam groups but is not visible in Figure 3D. The mAb 1611 (Figure 3D) was found to recognize only the $M_r \sim$ 50000 polypeptide in all foam groups, with no trace of binding evident to lower M_r polypeptides.

Detection of Barley Protein Z and β-Amylase in Lager Beer Foam Fractions. The binding to foam fractions by polyclonal antisera raised to barley protein Z and to β-amylase was determined by ELISA (Figure 4). Neither recognized the low MM fractions (data not

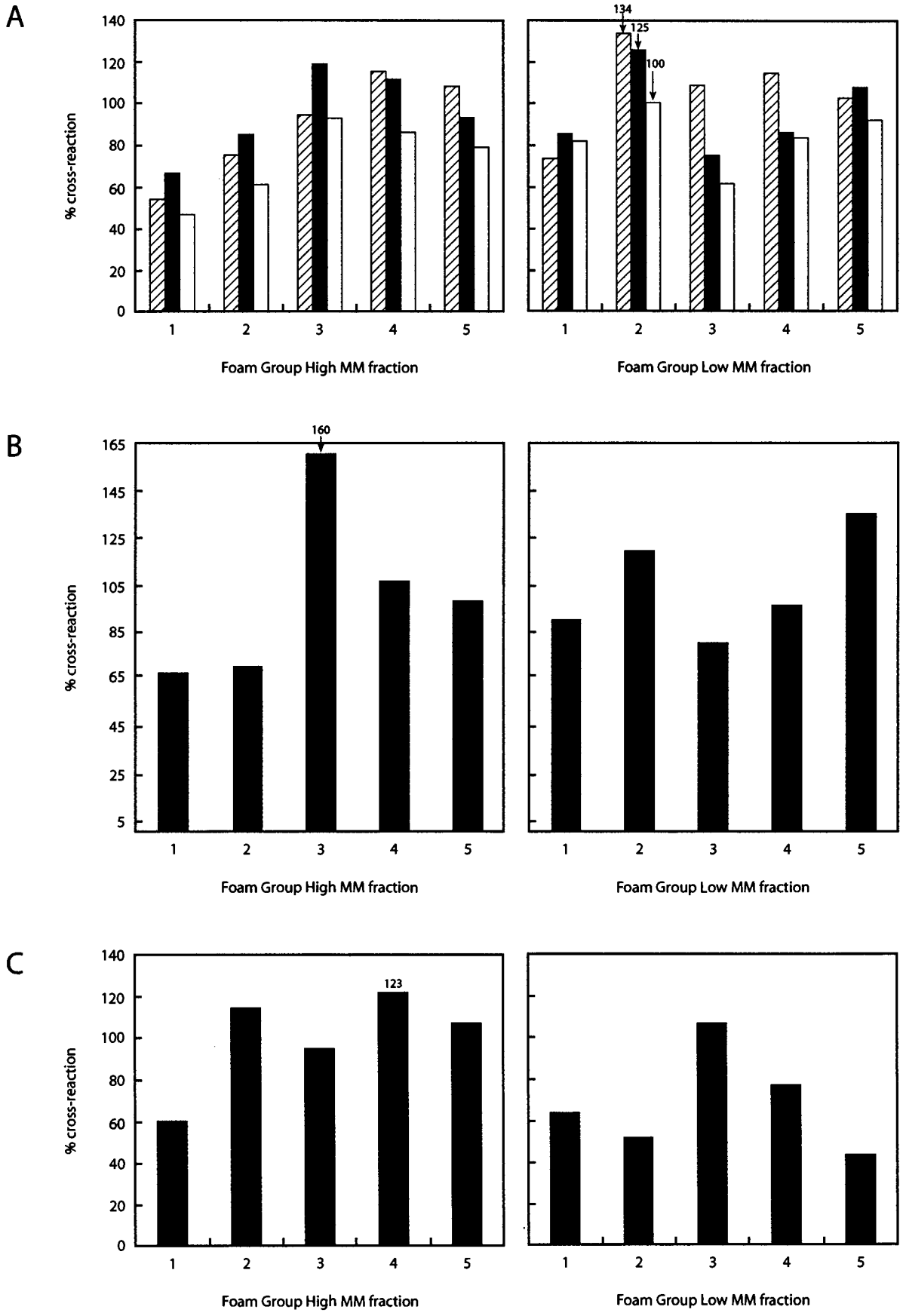
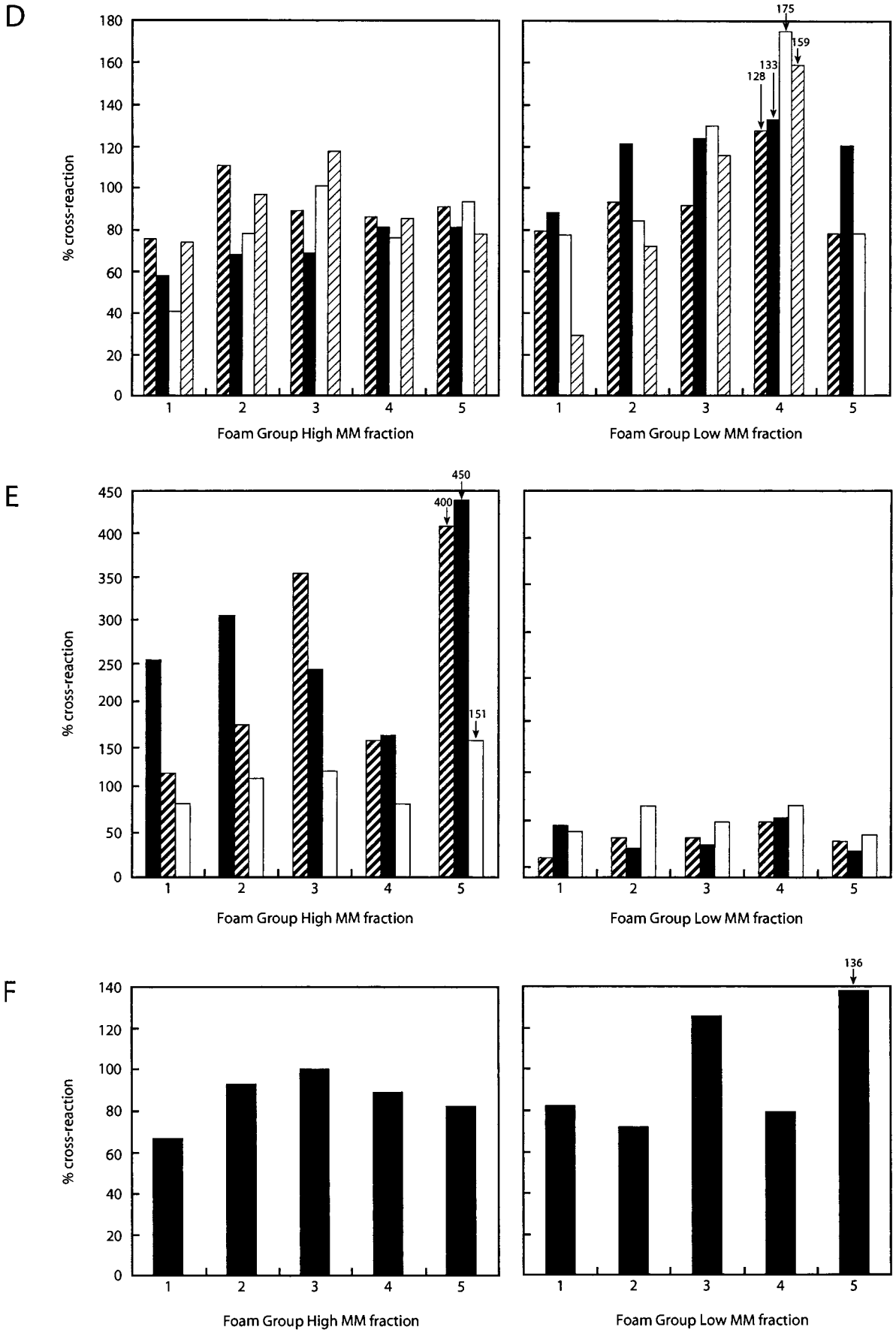


Figure 2. ELISA reactivity of selected anti-lager beer protein mAbs with Octyl-Sepharose fractions of lager beer foam. The binding of the anti-lager mAbs to Octyl-Sepharose fractions of lager beer foam, the hydrophobicity and foaming potential of which increased from group 1 through group 5, was assessed by ELISA. The effect of polypeptide size on mAb reactivity was also determined using high MM (>3000 Da) and low MM (<3000 Da) fractions of the foam groups. Cross-reactions are expressed as a percentage of the ratio of binding observed to each lager beer fraction to the binding observed to the 40% ammonium sulfate



(Figure 2, cont'd) fraction of lager beer, at a particular antibody dilution. Antibodies were as follows: (A) IFRN 1604 (lightface slashed bar), 1608 (black bar), 1619 (white bar); (B) IFRN 1605 (black bar); (C) IFRN 1611 (black bar); (D) IFRN 1615 (boldface slashed bar), 1618 (black bar), 1622 (white bar), 1623 (lightface slashed bar); (E) IFRN 1612 (boldface slashed bar), 1613 (black bar), 1620 (white bar); F, 1621 (black bar).

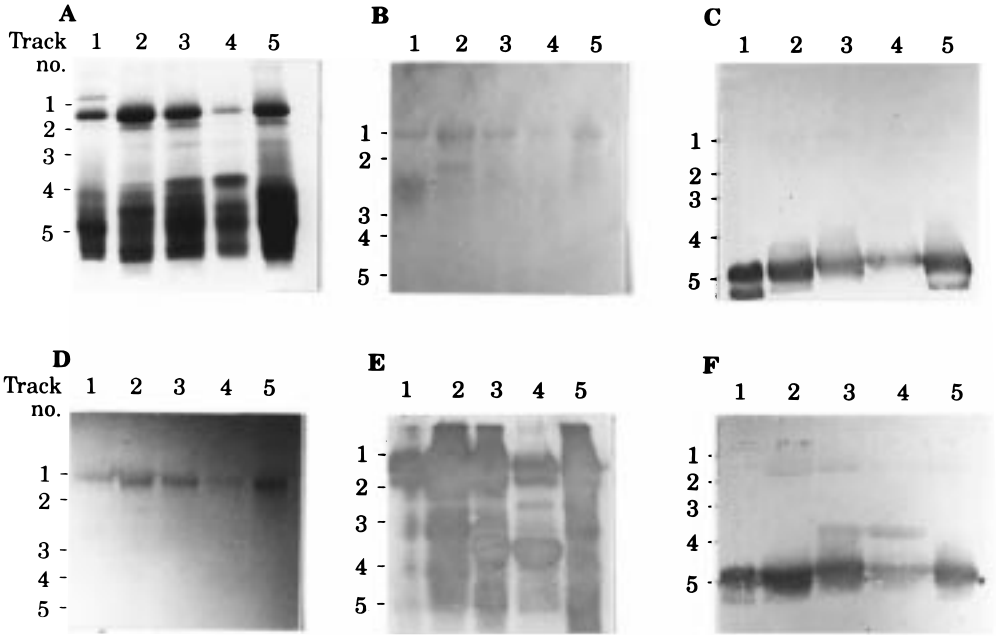


Figure 3. SDS−PAGE and immunoblotting analysis of the specificity of selected anti-lager beer mAbs, polyclonal anti-protein Z, and anti-β-amylase antisera toward Octyl-Sepharose fractions of lager beer foam. Tracks 1−5 were lager beer foam groups 1−5. Prestained molecular weight markers were as follows: 1, ovalbumin (49500); 2, carbonic anhydrase (34800); 3, soybean trypsin inhibitor (28300); 4, lysozyme (20400); 5, aprotinin (7200). A, Coomassie Blue protein stained gel; B−F immunoblots developed with mAbs IFRN 1612 (B), 1613 (C), and 1611 (D) together with polyclonal antisera against protein Z (E) and β-amylase (F).

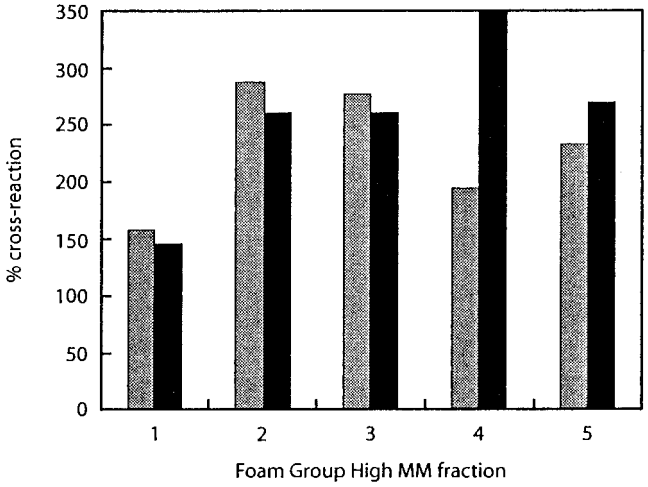


Figure 4. Detection of β-amylase and protein Z in lager beer foam fraction by ELISA using polyclonal anti-β-amylase and anti-Z antisera: (gray bar) protein Z; (black bar) β-amylase. Cross-reactions are expressed as a percentage of the ratio of binding observed to each lager beer fraction to the binding observed to a salt extract of barley, cv. Blenheim, at saturating levels of antibody binding.

shown), and both had the lowest cross-reactivity with group 1 polypeptides. The greatest amounts of immunoreactive protein Z were present in the high MM polypeptides of foam groups 2 and 3, with lower levels being present in groups 4 and 5. A different pattern was observed for β-amylase, the amounts of immunoreactive enzyme or derived fragments being greater in group 4 high MM fraction, with lower levels being present in groups 2, 3, and 5.

On immunoblots the polyclonal anti-protein Z antibody (Figure 3E) bound to an $M_r \sim 40000$ polypeptide present in all five foam groups, with a smear of material running the length of the gel being recognized in groups 2, 3, and 5 (tracks 2, 3, and 5, respectively). The antiserum also recognized a polypeptide of $M_r \sim 22000$ in foam groups 3 and 4. The anti-β-amylase antiserum (Figure 3F) recognized a single polypeptide of $M_r \sim 8000$ in all foam groups, albeit much more weakly in foam group 4 (track 4)]. Faint binding to a higher M_r polypeptide was also evident in foam groups 3 and 4 (Figure 3F).

Detection of Lager Beer Polypeptide Precursors in Malt. To determine the origin of the lager beer polypeptides recognized by the mAbs, the reactivity of these antibodies with protein fractions from the two types of malt used in the production of the lager beer was assessed. The majority malt was a pilsner-type, prepared by heating at temperatures of ~100 °C, whereas the minority malt, comprising 1−2% of the malt used for brewing lager, was a crystal-type, which had been prepared by roasting green malt at temperatures of 120−160 °C (Blenkinsop, 1991; Seaton, 1987). Two types of extract were prepared: (1) salt-soluble material, which would contain albumin/globulin-derived material, together with peptides resulting from the digestion of the storage proteins during the germination stage of malting, termed the salt fraction; (2) aqueous propanol-soluble material, which would contain all of the remaining storage proteins, termed the propanol fraction. SDS−PAGE analysis of these fractions from the pilsner malt showed the presence of a polypeptide corresponding in molecular weight to β-amylase together with a smear of material running at 24000−50000 Da and from 18000 Da to the dye front in the salt fraction (Figure 1B, track 1). The propanol fraction contained polypeptides that corresponded in size to D, C, B, and γ-hordeins present in the barley (Figure 1, tracks 2 and 3, respectively). Material from the crystal malt ran as a smear (data not shown).

From the data in Table 1, it can be seen that there was no relationship between the reactivity profiles of the mAb library toward lager beer foam groups and malt

Table 1. Cross-Reactivities of Anti-Lager Beer Protein mAbs with Salt- and Propanol-Soluble Proteins of a Pilsner (Majority) and a Crystal (Minority) Malt Determined by ELISA[a]

	% cross-reaction			
	majority malt		minority malt	
mAb	propanol fraction	salt fraction	propanol fraction	salt fraction
	Group 2 Low MM			
IFRN 1604	128	114	340	ND[b]
1608	100	100	100	50
1619	116	100	172	ND
	Group 3 High MM			
IFRN 1605	70	100	290	ND
	Group 4 High MM			
IFRN 1611	196	260	283	83
	Group 4 Low MM			
IFRN 1615	133	166	180	100
1618	100	ND	225	ND
1622	100	100	225	100
1623	80	140	412	375
	Group 5 High MM			
IFRN 1612	428	122	350	ND
1613	220	280	160	ND
1620	187	162	310	ND
	Group 5 Low MM			
IFRN 1621	100	100	150	ND

[a] Cross-reactions are expressed as a percentage of the ratio of binding observed to each malt fraction to the binding observed to the 40% ammonium sulfate fraction of lager beer, at a particular antibody dilution. [b] ND, no detectable binding.

fractions. For example, of the group 2 low MM antibodies, IFRN 1604 and 1619 both bound predominantly to the propanol fraction from the minor malt, whereas IFRN 1608 recognized the minor malt and both of the majority malt fractions equally well. The propanol fraction from both malts was recognized by all of the mAbs in the library. However, whereas the salt fraction of the major malt was recognized by all but one mAb (IFRN 1618), that of the minor malt was poorly recognize, with only six mAbs binding to it. The less extensively modified major malt was generally recognized by antibodies to the same extent as the lager beer fraction used for mAb production. The exceptions were IFRN 1612, which recognized the propanol fraction 4-fold better, and IFRN 1613/1611, which bound the propanol and salt fractions ~2−3-fold more strongly than the immunogen. Overall, the library of mAbs bound best to the propanol fraction of the minor malt, with 10 mAbs giving reactions of 2−4 times that obtained with the immunogen. One antibody, IFRN 1618, recognized only the propanol fraction from both malts, whereas another, IFRN 1623, bound primarily to the minor malt, recognizing the propanol and salt fractions almost equally well.

DISCUSSION

Whereas polyclonal antibody preparations have often been used in the study of beer polypeptides (Hollemans and Tonies, 1989; Mohan et al., 1992; Horiuchi et al., 1996; Hejgaard, 1977), this paper reports the production, for the first time, of a mAb library to beer proteins and polypeptides per se. The beer was fractionated with ammonium sulfate to produce an immunogen lacking in protein Z, a major barley albumin that is a major protein in beer (Hejgaard, 1977), and enriched in a range of other polypeptides which are not abundant in beer. This strategy was employed to maximize the chances of developing mAbs to other beer components and proved to be largely successful.

All of the anti-beer polypeptide mAbs (other than IFRN 1612 and 1613) recognized both high and low MM material in the foam groups, indicating that they were able to bind to epitopes present in small polypeptide fragments. These data contrast with those for the polyclonal antisera to protein Z and β-amylase, which did not recognize any low MM material. Similarly, a panel of anti-hordein mAbs used to probe Octyl-Sepharose lager beer foam fractions did not recognize the low MM material (<3000 Da in size) (Kauffman et al., 1994). This is consistent with general observations that have been made in the development of antibodies to proteins and peptides that (1) antibodies to small peptides are frequently able to recognize the same epitope contained in larger fragments but (2) those raised to intact proteins usually bind with lower affinity to small peptides, which represent only part of the contact region in the intact protein (Van Regenmortel, 1993). Thus, the extensive reactions of the mAb library with low molecular weight polypeptides of lager beer imply these were the main immunogenic species in the ammonium sulfate fraction used for mAb production.

Both the anti-lager beer mAbs and the polyclonal anti-protein Z and β-amylase antisera bound least well to the most hydrophilic foam group 1, as determined by ELISA. This fraction was similarly unreactive when probed with a panel of anti-hordein mAbs (Kauffman et al., 1994). Whereas it contains the majority of proteinaceous material from foam, this fraction also appears to comprise polypeptides with the least amount of tertiary structure, that is, existing in a more unfolded state, as indicated by dye binding (Onishi and Proudlove, 1994). Such features may be important in antibody recognition. Only mAbs 1612 and 1613 gave any marked differentiation between foam groups which related to their hydrophobicity, with the most hydrophobic foam group 5 being recognized most strongly by both these mAbs. This differentiation was lost on immunoblotting. One explanation for such observations is that proteins immobilized to the surface of microtitration plates retain some of the structural features involved in determining the surface active properties of foam group proteins. Thus, conformations adopted by the more hydrophilic components of lager beer foam may prevent, or reduce, mAb binding in the ELISA. On treatment with reducing agent and SDS prior to electrophoresis, these features would probably be lost, thus allowing mAb binding to all fractions equally well on the immunoblots. Such differences in presentation of epitopes by ELISA and immunoblots have been suggested to account for differences in antibody reactivity observed previously (Butler, 1993).

The beer polypeptides recognized by the mAb library were found to originate from the malts and, in many instances, were found in both the propanol- and salt-extractable material. SDS−PAGE analysis of the pilsner malt fractions indicated that, whereas the salt-soluble material was highly degraded, the propanol fraction contained clearly recognizable intact hordeins. The ELISA analysis of these fractions therefore suggests that the mAbs probably recognize hordeins and derived fragments which become salt-soluble after breakdown during the germination stage of malting. Although little

material was extracted from the crystal malt, presumably because of its more extensive chemical modification during higher kilning temperatures, it was still recognized by many of the mAbs. Indeed, in some instances (e.g., IFRN 1623) binding was much stronger than to the original beer fraction used for mAb production. This type of malt contains a much higher concentration of Maillard browning products than the beer or the pilsner malt (Moir, 1992; Seaton, 1987), and it may be that many of the mAbs actually recognize hordein-derived polypeptides which had been modified in this way. It has been found that pyrolysis products of sugars will react with proteins by combining with either amine-containing amino acids, such as lysine, or proline residues (Hiebl et al., 1987). In addition, a glycated form of a $M_r \sim 40000$ polypeptide termed SMB has been identified by Curioni et al. (1995), which these authors suggested arose as a result of Maillard reactions during the kilning stage of malting.

It is intriguing that IFRN 1612 bound strongly to both the propanol extracts of malt and the hydrophobic proteins of foam group 5 associated with foam-positive character (Onishi and Proudlove, 1994). These data indicate that the hydrophobic proteins are present at a high concentration in the malt and that these modified barley proteins present in malt make a significant contribution to the foam-positive polypeptides of beer. Protein Z and fragments of β-amylase were present to an equal extent in both the very hydrophilic foam group 1 and the highly hydrophobic foam group 5 fractions (of Table 1). Previous studies have shown immunologically reactive protein Z to be present in beer foam (Hejgaard and Sorensen, 1975), but these data suggest that, as protein Z is not present in increased amounts in the hydrophobic foam fractions, it does not make a major contribution to their enhanced foaming properties. In addition to surface active properties per se, it has been suggested that Maillard products may play a role in foam stabilization (Jackson and Wainright, 1978). The mAb probes described here offer the means of studying such aspects of foam in greater detail, particularly in identifying the barley proteins from which the foam-positive polypeptides of beer originate.

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