Monoclonal Antibody Probe for Assessing Beer Foam Stabilizing Proteins

Akiko Onishi,[†] Mike O. Proudlove,[†] Kamini Dickie,[†] E. N. Clare Mills,*[,‡] Juliet A. Kauffman,[‡] and Mike R. A. Morgan[‡]

Brewing Research International, Nutfield, Redhill, Surrey RH1 4HY, United Kingdom, and Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom

A monoclonal antibody (Mab; IFRN 1625) has been produced, which is specific for the most hydrophobic polypeptides responsible for foam stabilization. The binding characteristics of the Mab suggest that it is the conformation of certain hydrophobic polypeptides which is important for foam stabilization. An enzyme-linked immunosorbent assay (ELISA) for assessing the foam-positive form of the foam-stabilizing polypeptides in beer was developed using IFRN 1625. A good correlation was obtained between ELISA determination of foam-stabilizing polypeptides and an empirical means of determining foaming, that is, the Rudin head retention values, for a collection of beers of various foam qualities. Application of the ELISA to different stages of the brewing process showed that the amounts of foam-positive polypeptides increased during barley germination. During the brewing process the proportion of foam-positive polypeptides present after fermentation increased slightly, although a large amount was lost along with other beer proteins during subsequent steps, such as filtering. The present study demonstrates that the amounts of beer polypeptide present in a foam-positive form have a direct relationship with the foaming potential of beer, that their levels are altered by processing, and that there is potential for greater quality control.

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

INTRODUCTION

Brewing is one of the oldest applications of biotechnology and was evidently used by the Egyptian and Babylonian civilizations (Michel et al., 1992). However, it was very much an art, with the various steps involved shrouded in mystery. In modern times, the fermentation process was one of the first of these steps to be investigated in a systematic manner, with scientists working toward improving brewing technology and the quality of the end-product. The color, clarity, ability to form a stable head of foam, and flavor of beer are all important factors in determining the consumer's judgment of beer quality (Bamforth, 1985). Among these, the achievement of an attractive head of foam is the hardest to control consistently and is affected by a number of components including lipids, hop acids, polyphenols, and, most importantly, proteins. Beer proteins and polypeptides associate with carbohydrates, metal ions, and isohumulones derived from hops, to form the network of bubbles that comprise a stable head of beer. Both the size and surface-active properties of beer proteins are important in determining their behavior at the bubble surface, and the complexities of their interactions (with each other and other components) contribute to the difficulties in controlling foam quality. To identify the characteristics important in foam stabilization, beer foam proteins have been fractionated on the basis of their surface hydrophobicity (Onishi and Proudlove, 1994). A positive correlation between hydrophobicity of beer foam proteins and foam stability has been demonstrated, the absolute level of hydrophobic proteins being an important factor for beer foam stability. The proteins and polypeptides are derived primarily from malt, comprising storage proteins (Kauffman et al., 1994; Dale et al., 1989) and albumins (of which protein Z is the best characterized) (Hejgaard, 1977). Many have undergone extensive modification, including proteolysis and thermal denaturation during the malting and brewing processes, to produce the spectrum of polypeptides ranging from a few thousand daltons to 40000 Da in size found in beer (Onishi and Proudlove, 1994).

Many techniques have been developed for beer foam quality assurance, such as the Rudin assay (Rudin, 1957) and the Ross and Clark method (Ross and Clark, 1939). These methods are empirical and time-consuming to perform but are currently the most reliable way of assessing beer foam quality. In addition, such methods can be applied only to the final beer and are not suitable tools for process control. Techniques for monitoring hydrophobic foam-stabilizing proteins during processes such as malting and brewing would offer a new means of predicting foam quality. As such, these methods also have a role to play in improving the brewer's control over the brewing process. Immunological techniques have been previously applied to problems in the brewing industry using either polyclonal antibodies to beer proteins (Hejgaard, 1977; Ishibashi et al., 1996) or monoclonal antibodies (Mab) to barley proteins (Kauffman et al., 1994; Sheehan and Skerritt, 1997) and beer proteins (Mills et al., 1998). Furthermore, an immunoassay has been reported using a polyclonal antibody preparation to show that the level of a 40000 Da protein

* Author to whom correspondence should be addressed (fax 44-1603-507723; e-mail Clare.Mills@bbsrc.ac.uk).
[†] Brewing Research International.
[‡] Institute of Food Research.

10.1021/jf9810968 CCC: $18.00
Published on Web 07/29/1999

of beer was correlated with foam stability in beer (Horiuchi et al., 1994). This paper describes the production and characterization of a Mab to beer foam-stabilizing proteins, that is, the most hydrophobic beer foam protein fraction. One of these, IFRN 1625, was selected to develop an enzyme-linked immunosorbent assay (ELISA), which has been employed to determine the effects of malting and brewing on beer foam-stabilizing protein.

MATERIALS AND METHODS

Materials. BCA protein assay kit was from Pierce and Warriner (Chester, U.K.). Nunc Maxisorb 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-mouse IgM 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 Hempstead, U.K.). All other reagents were of AR grade.

Preparation of Beer and Malt Samples. Total beer protein fraction (TPF) was prepared by ultrafiltration of a Pilsner-type lager using a 3000 Da molecular weight cutoff ultrafiltration membrane in 50 mM dimethylglutarate, pH 4.5 (DMG), buffer containing 3.4% (v/v) ethanol. Foam polypeptides were separated into five fractions (groups 1−5) by hydrophobic interaction chromatography on octyl-Sepharose CL-4B. Group 1 was the most hydrophilic and group 5 the most hydrophobic fraction (Onishi and Proudlove, 1994). These fractions were further separated into high and low molecular mass fraction (HMM and LMM, respectively) by ultrafiltration using a 3000 Da cutoff membrane in DMG buffer containing 3.4% (v/v) ethanol. Fractions were stored in aliquots at −20 °C.

Malt samples were prepared on a 50 kg scale using a pilot-scale malting. A steeping regime, using two cycles of a 7 h immersion followed by a 17 h air rest followed by a 1 h immersion, was employed. At the end of the final 1 h steeping period, a sample was taken (termed casting) which was the equivalent of germination day zero (G0). Samples of grain were taken at 24 h intervals (G1−G4) during grain germination, which was performed at 16 °C. The final malt was the G4 grain, which had been dried at a final curing temperature of 85 °C for 26 h. Samples (0.5 g) of barley and malt (cv. Blenheim) were extracted in 5 mL of water for 1 h at 65 °C in a shaking water bath. This represents a liquor/grist ratio of 1:10 (v/w). Samples were centrifuged at 14000 rpm in a microcentrifuge for 5 min at room temperature and the supernatants removed for further analysis.

Samples (100 mL) were taken from a pilot-scale brew prepared using a liquor/grist ratio of 3:1 (v/w) at each of the following stages: wort boiling (90 min wort boil); post-whirlpool (wort was centrifuged to remove precipitated and aggregated material and allowed to settle for 20 min); start of fermentation (days 0−6 held at 12 ± 1 °C); end of fermentation (warm maturation, held at 13 °C ± 1 °C for 3 days); cold conditioning (held at 3 ± 1 °C for 7 days); start of stabilization (maturation in a bright beer tank); end of stabilization; prior to filtration; post-filtration; packaged and carbonated beer. Protein contents of barley and malt extracts, together with beer samples, were determined according to the bicinchoninic acid (BCA) method (Pierce, Rockford, IL) using BSA as a standard protein unless otherwise specified.

Antibody Preparations. Mab IFRN 1625 was produced essentially as described by Kohler and Milstein (1975) with the modifications of Mills et al. (1990). Mice were immunized with foam group 5 and Mabs selected on the basis of their binding primarily to this foam fraction, rather than the less hydrophobic groups. All cell culture procedures were performed using OptiMEM (Gibco, UK Ltd.) supplemented with 4% (v/v) fetal calf serum (FCS, from Advanced Protein Products Ltd., UK) as the culture medium. Mabs were isotyped using an isotyping kit (Sigma Chemical Co., Poole, U.K.), and culture supernatants were concentrated as required using Centriprep centrifugal concentrators (30000 Da cutoff membrane; Amicon, Stonehouse, U.K.). The characteristics of the anti-hordein Mab IFRN 0670, which recognizes B and γ-hordeins in barley and all foam groups equally well, have been described previously (Kauffman et al., 1994).

Immunoassay Procedures. *(1) Coating of Microtitration Plates.* For selection of Mabs, microtitration plates were coated (inner 60 wells only) with 0.3 mL/well of foam group 5 (1 μg of protein/mL) in 0.05 M sodium carbonate−bicarbonate buffer, pH 9.6 (coating buffer). After incubation overnight at 4 °C, plates were washed three times with water (using a Wellwash 5000, Life Sciences International UK Ltd., Billingshurst, U.K.), allowed to dry in air, and stored at −20 °C until required. For cross-reactions with different foam fractions, plates were coated using a Biomek 1000 (Beckman Instruments, High Wycombe, U.K.) with a different fraction (at 5 μg of protein/mL) in each quarter, the lower right quarter always being coated with foam group 5 HMM. The effect of coating conditions was examined using foam groups coated at 1 μg of protein/mL in either DMG buffer, PBS (0.14 M NaCl, 0.0015 M KH_2PO_4, 0.008 M Na_2HPO_4, 0.0027 M KCl; pH 7.4), or coating buffer. Barley and malt extracts were diluted to 300 μg of protein/mL, whereas beer samples were diluted to 100 μg of protein/mL in carbonate buffer and triplicate wells coated as described above. Amounts of IFRN 1625-reactive hydrophobic proteins in brewing process samples were determined using a standard curve constructed by coating foam group 5 proteins at 0.01−1 μg of protein/mL in coating buffer.

(2) Immunoassay Conditions. 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; PBS containing 0.05% (v/v) Tween 20] prior to the addition of 0.2 mL/well of anti-mouse IgM 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 TMB (Vetoquinol, Bicester, U.K.). 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 culture supernatant diluted appropriately in PBST; plates were then developed as described above. In this way comparable titration curves could be obtained for all fractions. Cross-reactivities (as a percentage) were calculated as the ratio of binding observed to each foam fraction to the binding observed to foam group 5 at a particular antibody dilution.

For the determination of IFRN 1625 reactivity with beer and malt samples, the average absorbance of each sample determined in the ELISA was expressed as a ratio of the protein content of the sample. Samples were coated onto microtitration plates at the following protein levels: beers and brewing samples, 100 μg/well; barley and malt extracts, 300 μg/well. For the brewing samples, the amounts of IFRN 1625-reactive hydrophobic protein were calculated for each sample from a calibration curve constructed by coating foam group 5 at 0.01−1 μg/mL. The detailed procedure for the characterization was as described by Kauffman et al. (1994).

Electrophoretic and Immunoblotting Procedures. Foam fractions, malt, and beer samples were separated using a 15% polyacrylamide gel Laemmli system (Onishi and Proudlove, 1994) with prestained molecular weight markers (Bio-Rad). Immunoblotting was performed essentially as described by Mills et al. (1990) using 0.2 μm pore nitrocellulose membrane

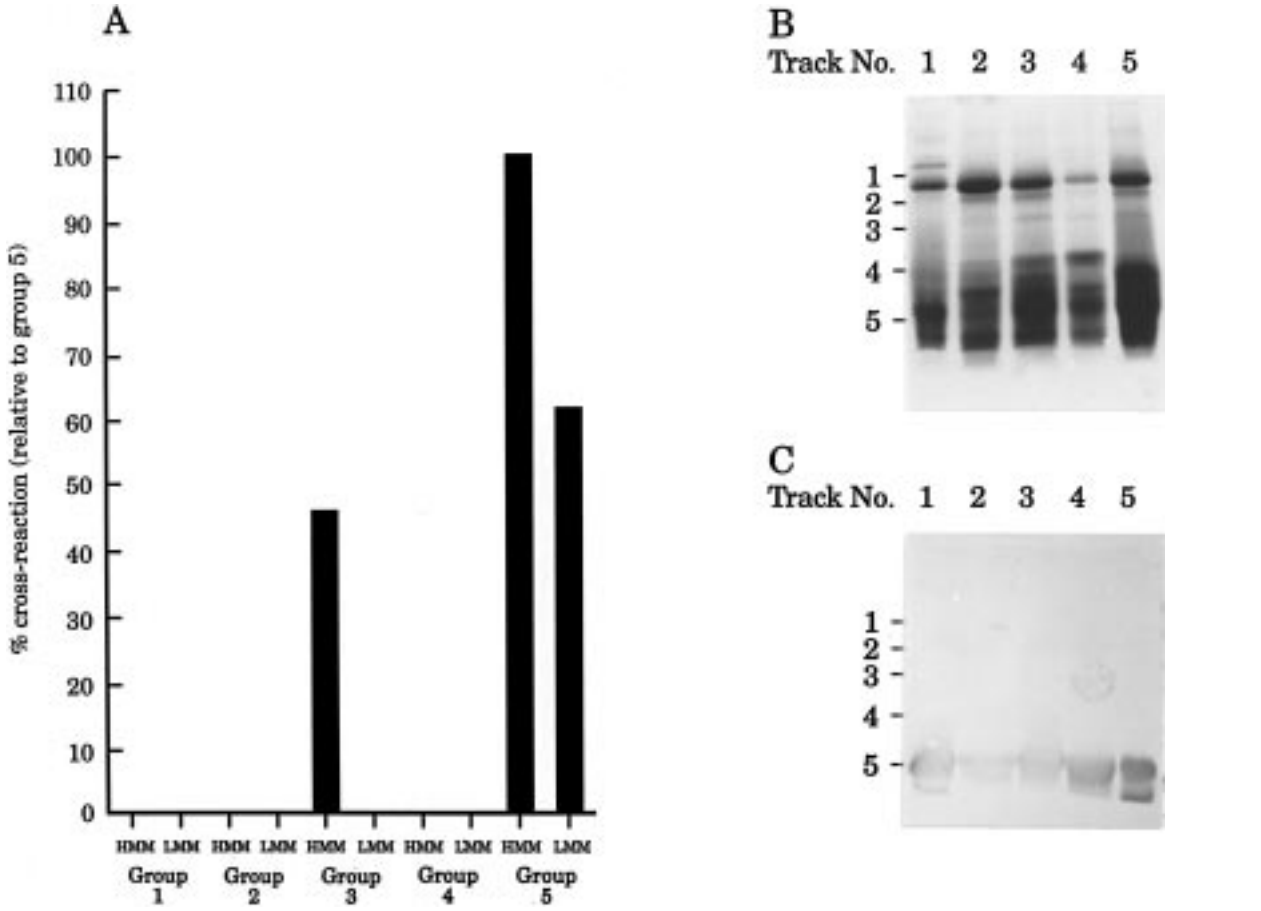

Figure 1. Cross-reactivity of IFRN 1625 with different beer foam fractions: (A) binding to foam fractions of increasing hydrophobicity from groups 1 through 5 was determined by ELISA; (B) SDS−PAGE of beer foam fractions; (C) corresponding immunoblot developed with IFRN 1625. Lanes were as follows: 1, group 1; 2, group 2; 3, group 3; 4, group 4; 5, group 5. Prestained molecular weight markers were as follows: 1, 45.0 kDa; 2, 36.0 kDa; 3, 26.9 kDa; 4, 18.1 kDa; 5, 7.7 kDa.

and an alkaline phosphatase/BCIP-nitroblue tetrazolium detection method.

Measurement of Foam Stability. The foam stability of each HMM fraction of the foam groups (i.e., with an $M_r > 3000$) was determined using a micro-Rudin apparatus (Rudin, 1957). The foam stability of each beer (Pilsner-type lager) was carried out using a Rudin apparatus and CO_2 as a foam-generating gas (Ross and Clark, 1939).

RESULTS

Identification of a Mab to Foam-Positive Polypeptides of Beer. A Mab, IFRN 1625, was raised to the most hydrophobic octyl-Sepharose fraction of beer, foam group 5 (Onishi and Proudlove, 1994). Figure 1A shows the cross-reactivity of this Mab with foam fractions, the hydrophobicity of which increases from group 1 through group 5, determined by ELISA using 0.05 M sodium carbonate−bicarbonate buffer, pH 9.6, for coating microtitration plates. A 50% cross-reaction was seen toward the HMM fraction (MW > 3000 Da) of foam group 3, although the LMM fraction (MW < 3000 Da) of this foam group was not recognized at all by IFRN 1625. The strongest binding was observed toward the immunogen, that is, foam group 5, with binding being slightly reduced toward the LMM fraction, which gave a cross-reaction of ~60%. When 50 mM DMG, pH 4.5, was employed for coating foam groups, Mab binding was lost, whereas when PBS was used for coating, the Mab binding was weaker and the differentiation of foam groups was lost (data not shown).

A different pattern of binding was obtained toward the foam groups by immunoblotting. Figure 1B shows a protein-stained gel of the foam groups showing that they all contained a dominant band of $M_r \sim 40000$, which has been shown to comprise protein Z (Mills et al., 1998). Most of the stained material had a $M_r \sim 18000$ or less, running as a complex pattern of polypeptides, with subtle differences in the patterns being evident among the groups. From the immunoblot (Figure 1C) it was found that IFRN 1625 recognized the same polypeptide ($M_r \sim 8000$ Da) in all of the foam groups. A trace of binding to some lower molecular weight material at the dye front was also evident.

The relationship between Mab binding and foaming ability of a collection of commercially produced beers was then assessed by ELISA (Figure 2A). A correlation between the degree of IFRN 1625 binding (as indicated by the absorbance value at 450 nm obtained in the ELISA) and foaming characteristics, as expressed by the Rudin head retention value, was obtained with a correlation coefficient of 0.69. This correlation was found only when Mab binding was expressed per unit of protein in the beer sample, as protein content per se affects foaming potential. No correlation (coefficient of −0.131) was obtained when the same analysis was performed using the anti-hordein Mab, IFRN 0670 (Figure 2B), which has been shown previously to recognize all foam groups equally well (Kauffman et al., 1994).

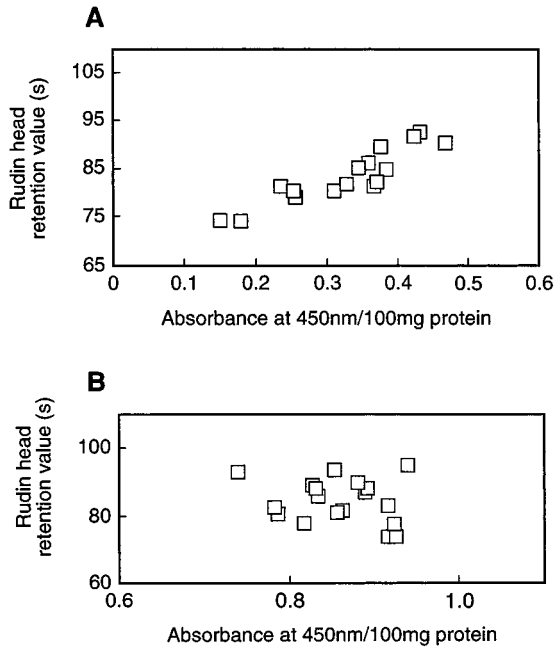

Figure 2. Relationship between foam stability of different beers and the amount of beer foam stabilizing proteins measured by ELISA using (A) anti-foam group 5 Mab IFRN 1625 and (B) anti-hordein Mab IFRN 0670. The binding of IFRN 1625 or 0670 to Pilsner-type lagers was determined by ELISA and plotted against foam stability assessed according to the method of Rudin (1957).

Changes in Foam-Positive Polypeptides during Malting and Brewing. An ELISA was then developed, employing the purified foam group 5 as a standard and Mab 1625, to quantify foam-positive barley and malt extracts taken throughout the malting process. Only a low level of binding was observed toward the barley extract, which increased during germination, reaching a peak at day 3 (Figure 3A). Interestingly, kilning (which takes place after day 4 of germination to give the final malt) had little effect on the amounts of IFRN 1625-reactive material. SDS−PAGE analysis of the malting samples showed there were extensive changes to the barley seed proteins, as indicated by the loss of hordein polypeptides, in particular the M_r = 60000 polypeptides corresponding to C hordein [cf. Figure 3B track 1 (barley) and track 6 (day 4 of germination)]. There was also a concomitant increase in the amounts of lower molecular weight polypeptides. As observed for the analysis of foam groups, the immunoblotting showed that the Mab recognized a low M_r polypeptide present in barley and at all stages of germination. Its relative mobility in the SDS−PAGE gel was unaffected by the malting process (Figure 3C).

The ELISA was then used to analyze the foam-positive polypeptides in samples taken throughout the brewing process (Figure 4A). During successive stages of the brewing process the foam-positive protein recognized by IFRN 1625 was lost. This was significant and progressive during fermentation (stages 1−4), with little change during the maturation stages 5−8. However, there was a further drop in foam-positive protein at stage 9, when the beer was filtered. SDS−PAGE followed by immunoblotting analysis (parts B and C of Figure 4, respectively) showed no change in the size of the polypeptide recognized by IFRN 1625 during the brewing process, although the levels of the polypeptide appeared to progressively decrease.

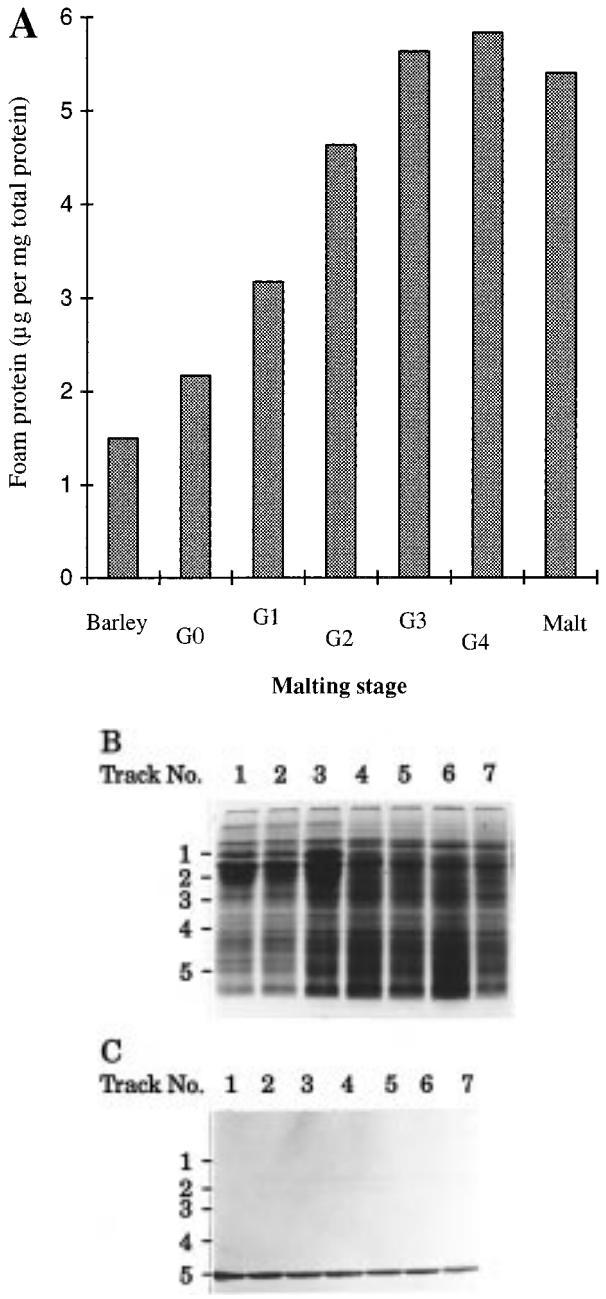

Figure 3. Effect of malting on levels of IFRN 1625-reactive foam-positive polypeptides: (A) analysis of barley and malting samples by ELISA using IFRN 1625 [Barley was cv. Blenheim, and samples were taken after casting (steeping; termed germination day zero G0) and at 24 h intervals (G1−G4) during grain germination, and after drying (malt)]; (B) 15% SDS−PAGE analysis of barley and malting samples; (C) corresponding immunoblot developed using IFRN 1625. Lanes were as follows: 1, barley; 2, G0; 3, G1; 4, G2; 5, G3; 6 G4; 7, malt. Prestained molecular weight markers were as described in Figure 1B.

Propyleneglycol alginate (PGA) is a high molecular weight carbohydrate isolated from kelp that is widely used as a foam stabilizer by the brewing industry. Its mechanism of action is not understood, although in a previous study it was found that PGA increased the hydrophobicity of hordein-derived fragments in beer (Kauffman et al., 1994). A total beer protein fraction (TPF) of a beer containing no PGA was analyzed for IFRN 1625-reactive polypeptides by ELISA before and after the addition of PGA (Figure 5). The binding of IFRN 1625 increased with increasing TPF concentra-

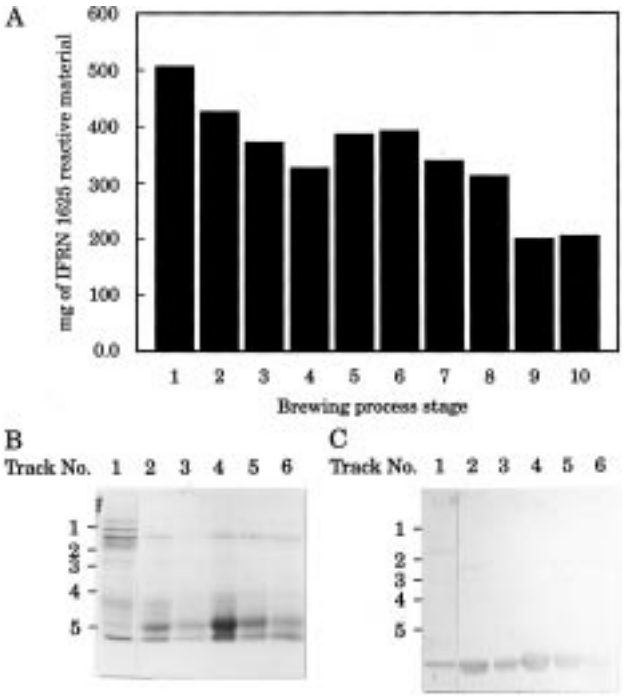

Figure 4. Effect of brewing on levels of IFRN 1625-reactive foam-positive polypeptides: (A) analysis of samples collected at different stages of the brewing process by ELISA (samples were as follows: 1, wort boiling; 2, post-whirlpool; 3, start of fermentation; 4, end of fermentation; 5, cold conditioning; 6, start of stabilization; 7, end of stabilization; 8, prior to filtration; 9, post-filtration; 10, packaged lager); (B) 15% SDS−PAGE of brewing samples; (C) corresponding immunoblot developed using IFRN 1625. Lanes were as follows: 1, total protein extract of the major malt used for the beer production; 2, after mashing; 3, wort boiling; 4, post-whirlpool; 5, end of fermentation; 6, packaged lager. Prestained molecular weight markers were as described for Figure 1B.

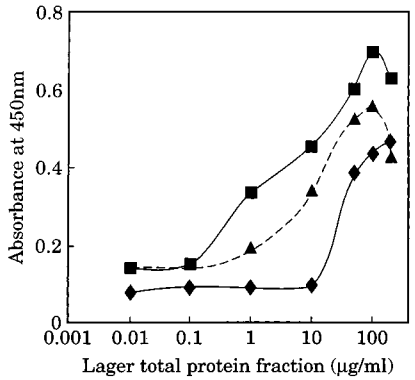

Figure 5. Effect of propyleneglycol alginate (PGA) on the binding of IFRN 1625 to total protein fraction (TPF) of beer: (■) TPF diluted in coating buffer alone; (▲) stock solution of 100 μg/mL of TPF mixed with 50 μg/mL of PGA and then diluted serially in coating buffer; (◆) TPF diluted serially in coating buffer containing100 μg/mL of PGA.

tion, reaching a peak of binding at 50 μg/mL of protein. The effect of altering the ratio of TPF to PGA was investigated by diluting TPF into PGA-containing buffer prior to coating ELISA plates. Mab binding was abolished at low protein concentrations (1−10 μg/mL) and reduced by ∼30% at higher protein concentrations (50−200 μg/mL). When the ratio of TPF to PGA was kept constant by the addition of PGA to a stock solution of TPF, followed by dilution in coating buffer alone, a less severe reduction in Mab binding was observed.

DISCUSSION

A Mab (IFRN 1625) has been developed that can bind selectively to the form of a polypeptide (M_r ∼ 8000) primarily present in the most hydrophobic fraction of beer foam, group 5. However, this selectivity was dependent on the way in which the protein was presented for Mab binding. Thus, both the pH and temperature employed for adsorption of the foam group polypeptides to microtitration plates were found to be important in retaining the selectivity of Mab binding (data not shown). Furthermore, the binding was indiscriminate after the foam groups had been subjected to denaturing electrophoresis. The selectivity of the Mab could be explained if it recognizes one of a number of forms of the 8000 Da polypeptide found in beer, which is characterized by being highly hydrophobic. Such phenomena have been observed previously (Butler, 1993), and other workers (Bech et al., 1995) have demonstrated that the denatured form of a barley lipid-transfer protein (BLTP) was important in determining foam stability. BLTP has an apparent molecular weight of ∼8000 Da, and it maybe that IFRN 1625 is recognizing this or a related polypeptide. Earlier studies have shown the compositions of each foam group to be very similar, as determined by SDS−PAGE and immunoblotting with anti-hordein Mabs (Onishi and Proudlove, 1994; Kauffman et al., 1994). It is likely that the different hydrophobicities of the various foam groups arise from differences in protein conformation and maybe that the conditions employed for adsorption of foam groups to microtitration plates retain the IFRN 1625-reactive polypeptide in a conformation related to its surface-active properties in beer.

The correlation between IFRN 1625 binding and foam head retention further indicates how the form of a polypeptide can determine its hydrophobicity and hence foaming properties. Although the protein content of beer is positively correlated with foam quality (Yokoi et al., 1989), this was taken into account by diluting the beers to the same protein concentration prior to assay. No correlation was obtained with the anti-hordein Mab 0670, which does not discriminate between foam groups. Thus, the amount of the IFRN 1625-reactive form of the 8000 Da polypeptide present in beer is positively correlated with desirable foaming properties. The IFRN 1625-reactive foam-positive polypeptide was found to originate in barley, and its levels increased during the germination phase of the malting process. This did not appear to be due to net synthesis, or breakdown from a larger precursor, as shown by immunoblotting. Its basis may only be resolved once the nature and function of the IFRN 1625-reactive protein in barley has been clarified. The finding that the levels of IFRN 1625-reactive material increased during malting but decreased during the brewing process was unexpected. Losses in IFRN 1625-reactive material during brewing occurred at stages such as filtering, when there is known to be a general loss of beer protein. It therefore appears that the malting, rather than the brewing, process, is more important in optimizing the levels of foam-positive protein. This is rather suprising, as the heating processes used during the brewing procedure, rather than germination, would unfold the barley polypeptides and thus alter their surface active properties. Other workers have found similar correlations with foaming quality but in relation to the amounts of a 40000 Da protein in beer. Foam quality is a complex

attribute of beer and is affected by a number of factors such as alcohol and hop acids in addition to proteins. It may be that both the 40000 Da beer component and the 8000 Da polypeptide described here contribute to the foaming potential of beer.

The known foam-stabilizing agent (Bennett, 1993), PGA, interfered with Mab binding to the very hydrophobic form of the 8000 Da polypeptide, as determined in the ELISA. A similar reduction in binding to beer proteins was observed for the Mab IFRN 0624, which recognizes a trypsin/α-amylase inhibitor type protein in barley (Kauffman et al., 1994). Work on model systems has shown that PGA will complex with proteins via a mixture of electrostatic and hydrophobic interactions (Ahmed and Dickinson, 1990), which probably underlie its foam-stabilizing properties (Jackson et al., 1980). Therefore, PGA binding to beer protein may cause a loss of Mab recognition as a consequence of reducing epitope accessibility, by causing a conformational change in the protein such that it is no longer recognized by the Mab or by preventing the protein from adsorbing effectively to the microtitration plates.

The data presented here show how new insights can be gained into a complex system (i.e., beer foam) by the application of modern biotechnological methods. Mabs, such as the one described here, offer a powerful means of improving our understanding of how processing can affect those components in beer that are important determinants of foaming quality. Thus, IFRN 1625 has the potential to be used in the development of an immunoassay or as a biosensor for monitoring raw materials and the brewing process for optimal levels of foam-positive polypeptides. The protein composition of beer is complex, and it is likely that foam quality is determined by a number of components; hence, the assay described here complements that of other workers. Future research will focus on the application of the ELISA to the analysis of beers, both during production and of the final product itself, and identification of the barley polypeptide recognized by IFRN 1625.

ACKNOWLEDGMENT

We gratefully thank Drs. R. Sharpe and I. Ormrod of Whitbread PLC, Luton, U.K., for supplying samples. A.O. and M.O.P. thank the Director General of Brewing Research International for permission to publish this paper.

LITERATURE CITED

Ahmed, M.; Dickinson, E. Foaming of aqueous solutions of protein and propylene glycol alginate. *Food Hydrocolloids* **1990**, *4*, 395−402.

Bamforth, C. W. The foaming properties of beer. *J. Inst. Brew.* **1985**, *91*, 370−383.

Bech, L. M.; Vaag, P.; Heinemann, B.; Breddam, K. Throughout the brewing process barley lipid transfer protein 1 is transformed into a more foam-promoting form. *Proc. Congr. Eur. Brew. Conf. Lisbon* **1995**, 561−568.

Bennett, A. N. Propylene glycol alginate for the brewing industry. *Proc. Inst. Brew. Conv. (Central and South African Region)* **1993**, *4*, 185−193.

Butler, J. E. The behavior of antigens and antibodies immobilized on a solid phase. In *Structure of Antigens*; Van Regenmortel, M. H. V., Ed.; CRC Press: Boca Raton, FL, 1993; Vol. 2, pp 209−259.

Dale, C. J.; Young, T. W.; Brewer, S. Amino acid analysis of beer polypeptides. *J. Inst. Brew.* **1989**, *95*, 89−97.

Hejgaard, J. Origin of a dominant beer protein; immunochemical identity with a β-amylase associated protein from barley. *J. Inst. Brew.* **1977**, *83*, 94−96.

Horiuchi, S.; Kouno, K.; Yamashita, H.; Mori, T.; Yabuuchi, S. Determination of beer foam protein by ELISA (Enzyme-linked immunosorbent assay). *Proc. 23rd Conv. Inst. Brew. Asia Pacific Sect.* **1994**, 198.

Ishibashi, Y.; Terano, Y.; Fukui, N.; Kakui, T.; Kawasaki, S.; Nakatani, K. Development of a new method for determining beer foam and haze proteins by using the immunochemical method ELISA. *J. Am. Soc. Brew. Chem.* **1996**, *54*, 177−182.

Jackson, G.; Roberts, R. T.; Wainwright, T. Mechanism of beer foam stabilization by propylene glycol alginate. *J. Inst. Brew.* **1980**, *86*, 34−37.

Kauffman, J. A.; Mills, E. N. C.; Brett, G. M.; Fido, R. J.; Tatham, A. S.; Shewry, P. R.; Onishi, A.; Proudlove, M. O.; Morgan, M. R. A. Immunological characterization of Barley Polypeptides in Lager Foam. *J. Sci. Food Agric.* **1994**, *66*, 345−355.

Kohler, G.; Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **1975**, *256*, 495−497.

Michel, R. H.; McGovern, P. E.; Badler, V. R. Chemical evidence of ancient beer. *Nature* **1992**, *360*, 24.

Mills, E. N. C.; Burgess, S. R.; Tatham, A. S.; Shewry, P. R.; Morgan, M. R. A. Characterization of a panel of monoclonal anti-gliadin antibodies. *J. Cereal Sci.* **1990**, *11*, 89−101.

Mills, E. N. C.; Kauffman, J. A.; Morgan, M. R. A.; Field, J. M.; Hejgaard, J.; Proudlove, M. O.; Onishi, A. An immunological study of hydrophobic polypeptides in beer. *J. Agric. Food Chem.* **1998**, *46*, 4475−4483.

Onishi, A.; Proudlove, M. O. Isolation of beer foam polypeptides by hydrophobic interaction chromatography and their partial characterization. *J. Sci. Food Agric.* **1994**, *65*, 233−240.

Ross, S.; Clark, G. L. On the measurement of foam stability with special reference to beer. *Wallerstein Lab. Commun.* **1939**, *6*, 46−54.

Rudin, A. D. Measurement of the foam stability of beers. *J. Inst. Brew.* **1957**, *63*, 506−509.

Sheehan, M. C.; Skerritt, J. H. Identification and characterization of beer polypeptides derived from barley hordeins. *J. Inst. Brew.* **1997**, *103*, 297−306.

Yokoi, S.; Maeda, K.; Xiao, R.; Kamada, K.; Kamimura, M. Characterization of beer proteins responsible for foam in beer. *Proc. Congr. Eur. Brew. Conf. Madrid* **1989**, 593−600.

Received for review October 8, 1998. Revised manuscript received May 7, 1999. Accepted May 10, 1999. We gratefully acknowledge financial support from the LINK Agro-Food Quality Programme.

JF9810968