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IDENTIFICATION OF THE INTERFACIAL PROTEINS IN MEAT EMULSIONS

C J Brock and M B Enser

AFRC Institute of Food Research - Bristol Laboratory
Langford, Bristol, BS18 7DY, UK

SUMMARY

The interfacial proteins from a meat emulsion have been identified directly by adapting the methods used to purify myofibrillar proteins from muscle. The matrix material was separated from the emulsified fat particles of the meat emulsion by repeatedly dispersing the emulsion, first in a high-ionic-strength solution used to solubilise myosin and other myofibrillar proteins, and then in a low-ionic-strength actin-extraction buffer. Emulsified fat particles were harvested by differential centrifugation. The interfacial proteins were isolated from the matrix-free emulsified fat particles by extraction of the fat with organic solvent, and identified by sodium-dodecyl-sulphate 10%-polyacrylamide-gel electrophoresis.

The emulsified fat particles contained major protein components with apparent molecular weights of 205000, 140000 and 120000 in this system. The first of these was identified as myosin (heavy chains) from beef muscle, while the other two were unidentified proteins originating from pork back fat which make up only a small proportion of the total protein in the meat emulsion. Higher molecular weight proteins from both beef muscle and pork back fat were also present in small amounts.

INTRODUCTION

It is not known with certainty which proteins act as emulsifiers at the fat-water interface in meat emulsions, but several lines of evidence suggest that salt-soluble muscle proteins play a major role. When oil is emulsified with a mixture of salt-soluble and water-soluble muscle proteins, the aqueous phase is preferentially depleted of the salt-soluble proteins (Schut and Brouwer, 1971). Moreover the dependence on pH of the emulsifying capacity of crude meat extracts follows that of salt-soluble protein more closely than it does that of water-soluble proteins (Swift and Sulzbacher, 1963). Among the many proteins from muscle which make up the salt-soluble fraction, myosin has particularly good emulsifying properties, and so it has been suggested that it is the major interfacial protein (Schut, 1976).

Although the above evidence is persuasive, it is nevertheless indirect. Therefore we set out to isolate the emulsified fat particles from a meat emulsion and to identify the interfacial proteins directly. The procedures for creating a meat emulsion from muscle are similar to the first steps in the purification of myofibrillar proteins from muscle. Both involve mincing the tissue and the addition of high-ionic-strength salt solutions. The main differences are in the addition of fat and the extensive comminution that creates the emulsified fat particles in a meat emulsion. Therefore it seemed appropriate to use traditional protein biochemical techniques and approach the problem as if a lipid-associated protein were being isolated.

MATERIALS AND METHODS

Pork back fat and beef were gifts from the Carcass and Abattoir Division, AFRC Institute of Food Research, Bristol Laboratory, UK. All reagents were the AR grade of BDH Chemicals Ltd, Poole, UK. All solutions were prepared with glass-distilled water.

Meat emulsions had the following composition: 57.6% lean beef, 25% pork back fat, 15% water (added), 2% sodium chloride, 0.4% tetra-sodium pyrophosphate, 0.01% sodium nitrite. To prepare one 600 g batch, 345.6 g coarse-minced (7 mm) lean beef (prepared from muscle out of which the visible fat and connective tissue had been dissected), 150 g pork back fat, and 50 ml water containing 12 g sodium chloride, 0.6 g sodium nitrite in solution, all at 0°C, were added to 2.4 g tetra-sodium pyrophosphate dissolved in 40 ml water at room temperature, because of its limited solubility in the cold. These were comminuted for 90 s in a pre-chilled Robot Coupe R2 food processor. The temperature of the meat emulsion was checked, and samples were taken for pH determination, cooking, electrophoresis and a separate study by light microscopy. The remainder (>95% of the total) was dispersed into 2 l of a modified Guba-Straub solution (0.3 M potassium chloride, 0.1 M potassium dihydrogen orthophosphate, 0.05 M dipotassium hydrogen orthophosphate, 0.005% sodium azide) (Perry, 1955) at 4°C by forcing the emulsion through a 20 ml disposable syringe (without a needle) into the continuously-stirred solution. The emulsion was extracted by stirring at 4°C for 20 h. The fraction containing the emulsified fat particles was harvested by centrifugation at 2200 g for 1 h, under which conditions it floated on the bulk aqueous phase. It was dispersed twice more by stirring into 1.5 l Guba-Straub solution at 4°C for 22 h, and harvested as before. Samples were taken for electrophoresis and light microscopy. The material containing the emulsified fat particles was then dispersed in a low-ionic-strength actin-extraction buffer (2 mM Tris-chloride, 0.2 mM sodium adenosinetriphosphate, 0.5 mM dithiothreitol, 0.2 mM calcium chloride, 0.005% sodium azide, pH 8.0) (Pardee and Spudich, 1982) and harvested three times more as before. Samples were taken for electrophoresis and microscopy. Fat was extracted from all the samples taken for electrophoresis with chloroform:methanol (2:1). The insoluble material was collected, dried *in vacuo*, and its protein composition analysed by sodium-dodecyl-sulphate 10%-polyacrylamide-gel electrophoresis in a discontinuous buffer system (Laemmli, 1970).

RESULTS AND DISCUSSION

Microstructural studies of commercial frankfurters have shown that the highest proportion of truly emulsified fat is found in products in which the meat is highly comminuted (Theno and Schmidt, 1978). Therefore for this study the comminution time was chosen to be the longest that could be achieved without overprocessing. Overprocessing was judged to have taken place when the total weight loss (water and fat) after cooking the meat emulsion for 10 min at 90°C and draining exceeded 1% of its initial weight. The optimum comminution time was determined in preliminary experiments to be 90 s. Emulsions were smooth in texture, red-brown in colour, had a pH of 6.0-6.2 and a temperature no higher than 22°C after comminution. Shorter comminution times produced emulsions in which most of the fat was found as large pools in air vacuoles rather than being distributed throughout the matrix as fine particles. They were also coarse in texture due to the presence of large myofibrillar fragments.

The electrophoretic pattern of the meat emulsion (Fig 1, track 4) contained a number of bands in addition to those present in the beef muscle mince (track 2), the pattern of which was similar to those of muscle preparations published previously (Young and Davey, 1981, Starr and Offer, 1971). Of these additional proteins, major bands corresponding to molecular weights of 65000, 120000 and 140000, as well as some

minor ones of very high molecular weight (>205000), originated from the pork back fat (track 3). Other minor differences among the higher molecular weight proteins between the minced muscle and the meat emulsion are likely to be due to proteins which were more effectively extracted into the electrophoresis buffer from the highly comminuted meat emulsion than they were from minced muscle which remains highly structured. When minced muscle tissue or myofibrils are solubilised for SDS gel electrophoresis, using comparatively gentle comminution procedures, some insoluble material always remains (Young and Davey, 1981), and this is likely to comprise less-soluble high molecular weight structural proteins.

After the three washes with Guba-Straub solution the fraction which floated after centrifugation, and therefore contained emulsified fat, contained a lot of fibrous material. The whole fraction was now white. Electrophoresis of this material (Fig 1, track 5) showed that many proteins, particularly those of M_r less than 100000 had been partially or wholly extracted. The most abundant protein at this stage was myosin (M_r 205000). Of the other proteins that remained the two with molecular weights of 140000 and 120000 were enriched in this fraction relative to myosin. A significant amount of actin (which ran just ahead of the M_r 45000 marker) also remained, even though it was depleted relative to myosin compared with its abundance in the meat emulsion.

Washing with actin-extraction buffer resulted in separation during centrifugation of the emulsified fat particles, which floated, from the fibrous material, which sedimented. It was clear that the fat was still truly emulsified, because even after centrifugation at 2200 g it did not coalesce into a continuous fat phase, but dispersed readily into the aqueous phase as a fine suspension of discrete particles. Nevertheless, there was good evidence from light microscopy of stained sectioned samples that the bulk of the matrix material had been extracted. At all earlier stages of the preparation the region between the fat particles contained material which stained readily for protein. After the washes in actin-extraction buffer only fat particles could be detected in stained sectioned material in the light microscope, insufficient protein remaining to produce a positive reaction to protein stain (A Cousins and C Brock, unpublished results).

That the small amount of material remaining emulsifying the fat was protein was indicated by electrophoresis of the material precipitated after organic solvent extraction of the fat (Fig 1, track 6). Almost all the actin had been extracted by this stage, and in some preparations of emulsified fat particles it was not present at all. Major protein bands remained corresponding to molecular weights of 205000, 140000 and 120000, together with minor bands of higher molecular weight.

The M_r 205000 band corresponded to myosin (heavy chain), which was present among the standard protein markers. Myosin light chains ran at the front in this system and therefore were not resolved.

The M_r 140000 and 120000 bands corresponded to two of the unidentified proteins from pork back fat mentioned above. Their identity was confirmed in a separate experiment (data not shown) in which the proteins from pork back fat and emulsified fat particles were electrophoresed together in the same track. The abundance of these proteins in the interfacial films was surprising in view of the small proportion (1-2%) of the total protein in the meat emulsion which is contributed by the pork back fat. However it should not have been entirely suprising that some of the proteins in pork back fat were so lipophilic.

The possibility cannot be excluded that myofibrillar proteins contributed to these bands. The M_r 140000 band may include some C-protein, which is a major muscle protein with that molecular weight (Starr and Offer, 1971, Offer et al, 1973). C-protein is associated with myosin in the thick filaments of myofibrils.

The identity of the minor bands which run behind myosin is uncertain. The most prominent of these bands comes from the pork back fat. The others may be traces of very high molecular weight myofibrillar components such as titin or nebulin (Locker and Wild, 1986). It is interesting to note that all the proteins isolated with the emulsified fat particles, whether clearly identified or not, have molecular weights greater than 100000.

This study has confirmed that myosin is the most important emulsifying protein present in muscle. It has also shown that in real meat emulsions, lipophilic proteins associated with the added fat contribute significantly to the interfacial films even though they make up only a tiny proportion of the total protein present. These experiments show clearly the direction that future research should follow. The emulsifying proteins from pork back fat must be isolated and characterised to the point where they can be identified unequivocally. Other meat emulsions, containing muscle and fat from other sources, should be analysed in a similar way to determine the protein components in them, which, with myosin, are important in emulsification. From such information common structural features of emulsifying proteins may be identified, thus assisting the elucidation of the mechanism of fat emulsification by proteins.

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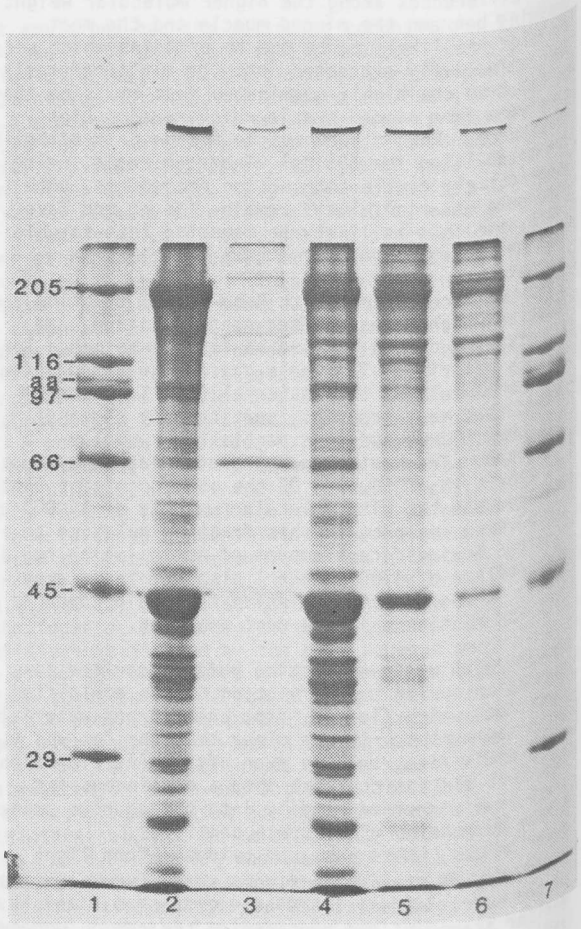

Figure 1

Electrophoresis of samples taken at various stages during the preparation of emulsified fat particles from a meat emulsion in a 10% polyacrylamide gel containing SDS (Laemmli, 1970). Tracks 1 & 7: standard protein markers (number = subunit molecular weight x 10^{-3}): 205; myosin (rabbit muscle), 116: β-galactosidase (E. coli), aa; α-actinin (chicken gizzard), 97; phosphorylase B (rabbit muscle), 66; albumin (bovine serum), 45; albumin (chicken egg), 29; carbonic anhydrase (bovine erythrocyte). Track 2: beef muscle mince. Track 3: Delipidated pork back fat. Track 4: meat emulsion. Track 5: fraction containing emulsified fat after meat emulsion had been extracted 3 times with Guba-Straub solution. Track 6: matrix-free emulsified fat particles after extraction 3 times with actin extraction buffer.