PROTEIN-LIPID AND PROTEIN-CARBOHYDRATE INTERACTIONS IN THE GLUTEN COMPLEX[1]

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Abstract

It is well known on the basis of earlier research and observations that defatting of flour or addition of polar lipids affect gluten proteins. More recent research made in Kansas, Winnipeg and also in our laboratory revealed the important role of phospho- and glycolipids in the interactions of proteins and lipids and in the formation of protein lipid aggregates. It was also stated that the formation of aggregates mentioned above is not a spontaneous effect but a result of specific interactions between lipids and given protein subunits. Good correlation was found between the polar lipid content and baking value of wheats resp. flours.

The role of carbohydrates in the formation of gluten complex has not been investigated so thoroughly as the lipid–protein interaction. Theoretically – due to the polar character of carbohydrates – hydrogen bonds may be formed between polar side chain groups of amino acid residues and hydroxyl groups of carbohydrates. A covalent binding of carbohydrates by O – or N – glycosidic bond is also possible. Ionic binding may not be excluded if oxidized carbohydrate derivatives are present. In our laboratory Örsi has found that some high molecular weight components of glutenin contain a significant quantity of carbohydrates.

Experiments made in our laboratory showed that some gel-forming polysaccharides containing ionic groups improve the dough properties and stability. The explanation of the character of interactions needs further investigations.

Keywords: gluten, protein–lipid interaction, protein–carbohydrate interaction, gluten complex.

Introduction

Although the most important role of two classical protein types of gluten – the gliadin and glutenin – in the formation of gluten complex is at present not opposed by specialists, nevertheless the deeper knowledge of the process of gluten formation and of factors influencing gluten properties needs an explanation of the role of other protein and non protein components of gluten.

The questions to be answered in relation to non protein components may be summarized as follows:

- Which non protein components are important?
- How do they interact with gluten protein?
- Is the role of these components significant?
- If yes, to what extent?

Wet gluten – depending on the conditions of preparation – always contains lipids and also carbohydrates. In view of this, first of all lipids and carbohydrates were considered as the main non protein components playing a role in the gluten formation. This was confirmed by some early experiments from which two very typical may be mentioned:

- defatting of flours makes the washing of gluten more difficult and changes the properties of gluten;
- in the presence of high amounts of rye polysaccharides, it is not possible to obtain gluten by dough washing.

Protein–Lipid Interactions

Concerning the form of interactions of proteins and lipids, theoretically four types of interactions are possible,

- covalent bonding;
- ionic bonding;
- hydrogen bonds;
- hydrophobic interactions.

The nature of the real interactions may be well studied using small proteins of low molecular weight as models. In our laboratory model experiments were made with purothionin and different polar lipids (BÉKÉS – SMIED, 1981).

Model solutions were prepared containing pure purothionin fraction, and different lipids (egg lecithin, L-alpha-dipalmitoyl lecithin, phosphatidyl-ethanolamine, phosphatidyl-choline, phosphatidyl-serine, phosphatidyl inositol, cholesterol, ergosterol-palmitate, monogalactosyl-diglyceride, digalactosyl-diglyceride). The bound lipid content and the lipid-protein complexes were studied. Chemically modified purothionins (basic side chains were masked using maleic acid or cyclohexanedion) were also used in model experiments. It was stated that the interaction between purothionin and phospholipids is the strongest. If the basic side chains were blocked, the binding of phospholipids decreased rapidly. This fact suggests the conclusion that the phospholipids are bound by side chains of arginine and

lysine. The model experiments have shown that glycolipids may be integrated in the complex only if the protein-phospholipid complex is formed. The data on the quantity of glycolipids to be bound suggest the formation of phospholipid-glycolipid bonds rather than protein glycolipid bonds. The occurrence of neutral lipids in some preparations might be interpreted as phospholipid (glycolipid)-neutral lipid interaction (not excluding the hydrophobic side chains of amino acid residues and neutral lipids). On the basis of these investigations a hypothetical lipopurothionin structure was proposed as it is shown in *Fig. 1*.
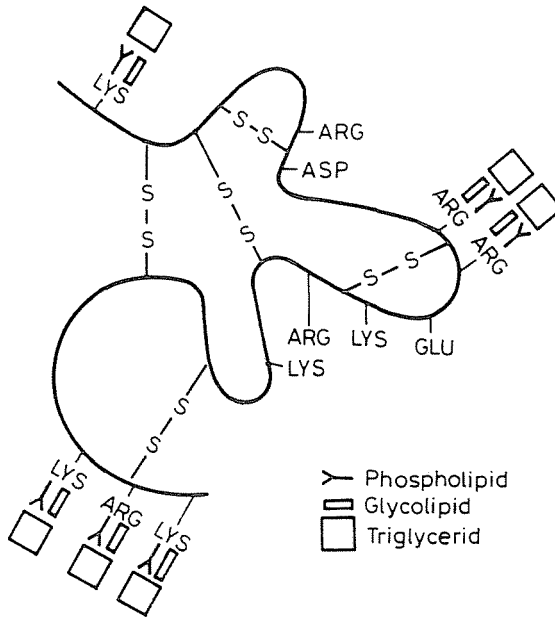

Fig. 1. Hypothetical structure of lipopurothionin (BÉKÉS – SMIED, 1981)

The next question which needs an answer in connection with protein–lipid interaction is: which wheat protein fractions interact with lipids? According to our present knowledge minimally four types of protein lipid complexes are present in native wheat grains (LÁSZTITY, 1996),

- complexes of low molecular weight gliadins (LMWG) with lipids;
- CM-(chloroform-methanol soluble) proteins (ligolin?, membrane proteins?);
- lipopurothionins;
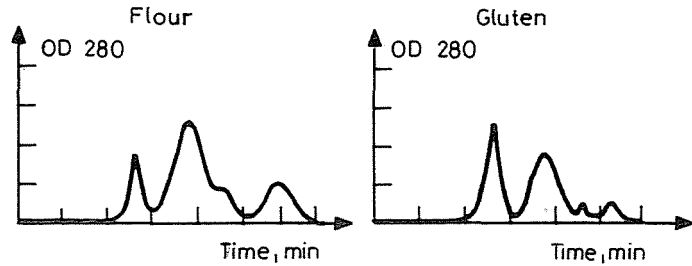- glutenin fractions?

Fig. 2. HPLC separation of 70% ethanol soluble wheat proteins: The effect of gluten
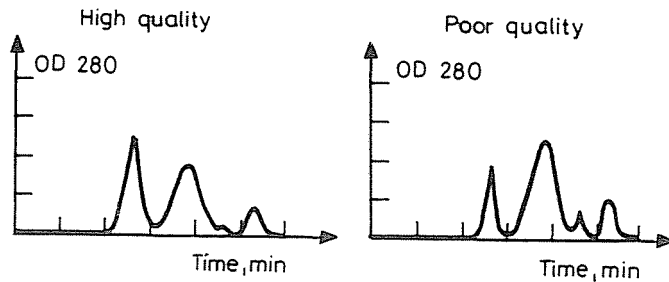washing

Fig. 3. HPLC separation of 70% ethanol soluble wheat gluten proteins. The effect of
baking quality

From the point of view of gluten formation the question is: which
gliadin resp. glutenin components (subunits) are capable of reacting with
lipids.

As it is generally known in the last 50 years a vast knowledge was
collected about the protein–lipid interaction of gluten proteins, a number
of different ideas were published concerning this topic. It was suggested
that the glutenin fraction has lipid binding capacity, others found a lipid
content of flour or gluten in the gliadin fraction. POMERANZ and his cowork-
ers (1980, 1983) in Manhattan, Kansas published a very useful model for
the characterization of protein–lipid interaction in gluten, saying that a
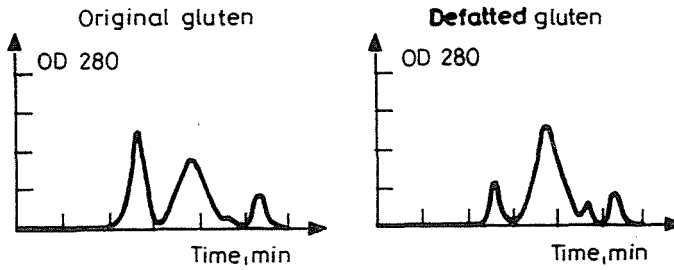
Fig. 4. HPLC separation of 70% ethanol soluble wheat gluten proteins isolated from high quality flour
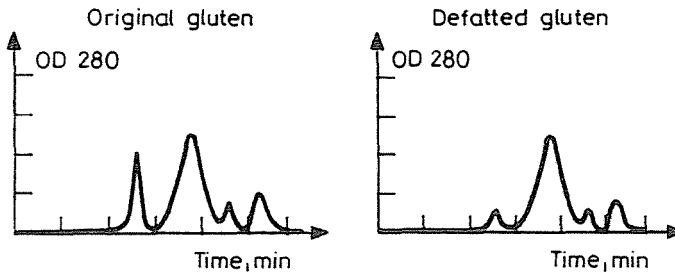

Fig. 5. HPLC separation of 70% ethanol soluble wheat gluten proteins, isolated from poor quality flour

glutenin–polar lipid–gliadin complex exists in the developed gluten caused by the wetting of flour and by the mechanical input during doughmaking.

Such complex was isolated in Bushuk's laboratory in · Winnipeg (Canada) from a 70% ethanolic extract of gluten (BÉKÉS et al., 1983, 1983a) containing HMW gliadin subunits. As it is known, HMW gliadins are chemically glutenin-like proteins, which, having interacted with polar lipids and LMW gliadin components, became soluble in ethanol.

This interpretation underlines the main reason for the confusion in this area. Depending on the procedure used to separate gliadin and glutenin we can find this complex – and of course the lipid content of gluten, either in the gliadin or in the glutenin fraction. If we use separation methods based
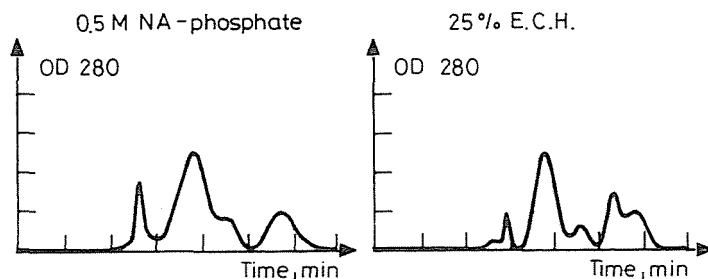
Fig. 6. HPLC separation of 70% ethanol soluble wheat. flour proteins. The effect of
    eluents

on solubility differences, lipids and the HMWG–lipid–LMWG complex goes
to the gliadin fraction, while using methods where the principle of separa-
tion is the molecular size difference (chromatography, ultracentrifugation),
lipids, in complex form, can be found in the glutenin fraction.

Let us summarize some characteristics of this protein-lipid complex,
based on our HPLC results in Budapest. Gel-filtration type separation
technique on HPLC results 4 – 5 fractions of 70% ethanol soluble gluten
proteins, very similar to those of Sephadex G-200 separation. The elution
profile and of course the distribution of proteins depends on the source
of·the samples, the pretreatment (namely on the mode of defatting) and
on the parameters of the separation. The effect of some parameters is
demonstrated in *Figs 2 – 6* showing the effect of the method of gluten
making, the effect of different baking strengths, the effect of defatting and
finally the effect of the eluent used.

Summarizing these results, we can say that the amount of excluded
fraction increases as caused by gluten making, but only in the case if lipids
are present in the flour. Defatting of gluten decreases the amount of ex-
cluded fractions but the level of the decrease is dependent on the baking
properties: a variety with better baking properties yields higher amount of
excluded fraction, and the better the baking properties the lower the effect
of defatting, thus the smaller decrease in the amount of excluded fractions.

The structure of the aggregate is quite unstable, and can be character-
ized using eluents with different pH and/or polarity in HPLC separations.

Low molecular weight gliadins form a very heterogeneous mixture.
We investigated whether LMW subunits can build into the lipid mediated
aggregate spontaneously or there are specific interactions between given
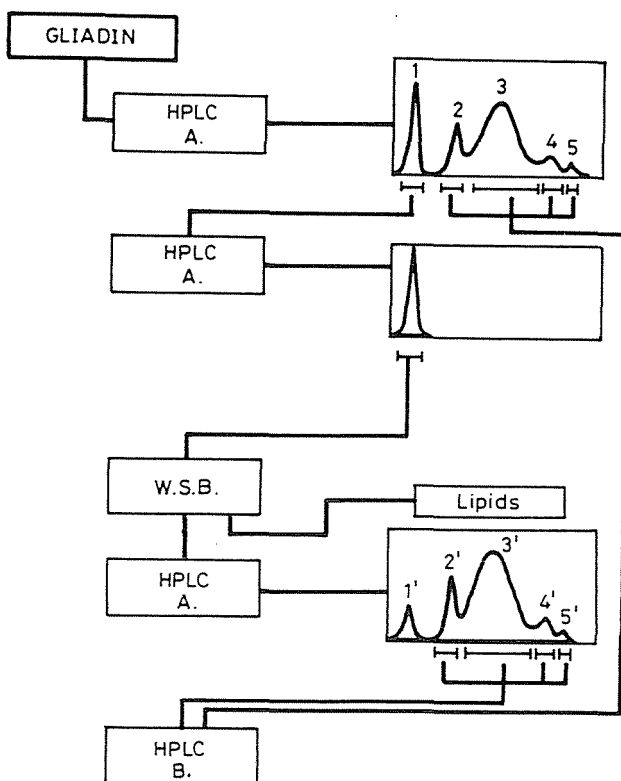gliadin subunits and lipids.

Fig. 7. Scheme of the procedure used for the investigation of HMWG–lipid–LMWG complexes

In this experiment two kinds of HPLC separations were combined in 4 steps. Gel-filtration-type separation was used to isolate the HMWG–lipid–LMWG complex. We made recirculation chromatography to isolate the purified excluded fractions without any contamination of lower molecular weight fractions. Then the purified aggregate was defatted with WSB and chromatographed again using the same conditions (see *Fig.* 7).

As you can see the complex fell apart yielding HMWG and LMWG fractions, with the same molecular weight as we found in the original gliadin.

These LMW fractions isolated from 70% ethanol soluble fraction of untreated and defatted gluten as well as from defatted aggregates were investigated by means of the method of Bietz, applying RP-HPLC. Some results are presented in *Figs 8 – 10.*
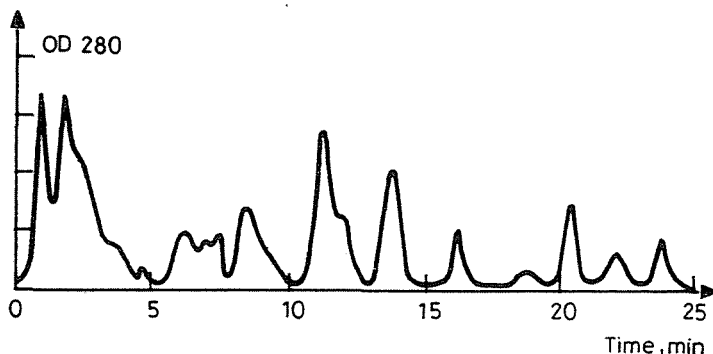
Fig. 8. RP-HPLC separation of LMWG fractions 2 and 3 (See *Fig. 7*) extracted from gluten. (Synchropak RP-P, 15-80% acetonitrile)
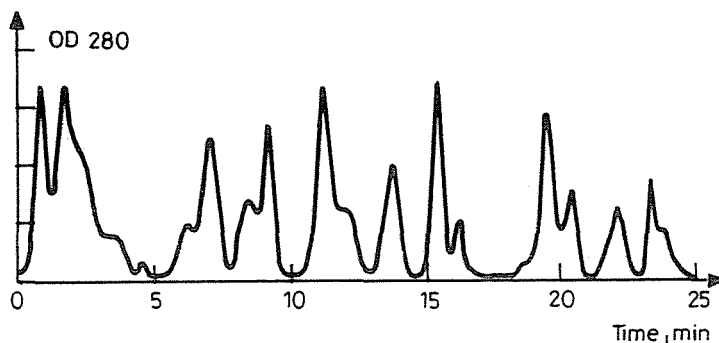

Fig. 9. RP-HPLC separation of LMWG fractions 2′ and 3′ extracted from defatted gluten (Synchropak RP-P, 15 – 80% acetonitrile)

As it can be seen, LMW subunits can be observed, which have significantly higher capability to be incorporated in the protein lipid complex, thus the formation of this aggregate is not a spontaneous effect but a result of specific interactions between lipids and given protein subunits.

Regarding the structure of complexes resp. the position of lipid molecules in the gluten complex two hypothetical possibilities are shown in *Figs 11 – 12*. In both variations the lipid molecules influence the aggregation of protein molecules and the formation of the network of gluten complex.
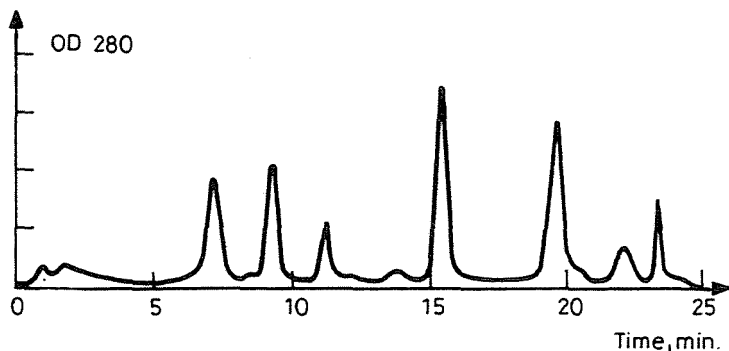
Fig. 10. RP-HPCL separation of LMWG fractions 2 and 3 isolated from defatted HMWG complex (Synchropak RP-P, 15 – 80% acetonitrile)
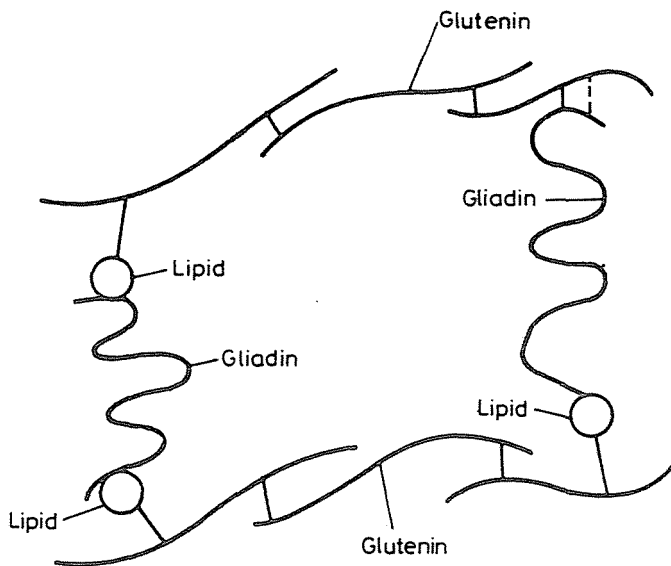

Fig. 11. Possible location of lipid molecules in the gluten network

Protein–Carbohydrate Interaction

Regarding carbohydrates and their role in the gluten complex our knowledge is rather limited. Due to the polar character of carbohydrate mole-
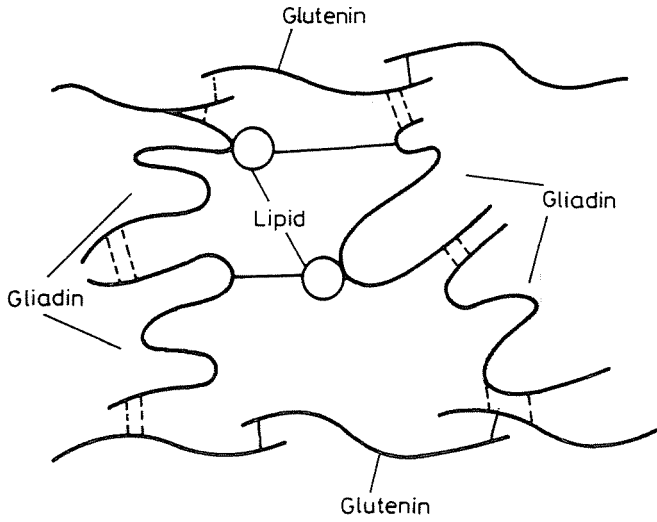
Fig. 12. Possible location of lipid molecules in the gluten network
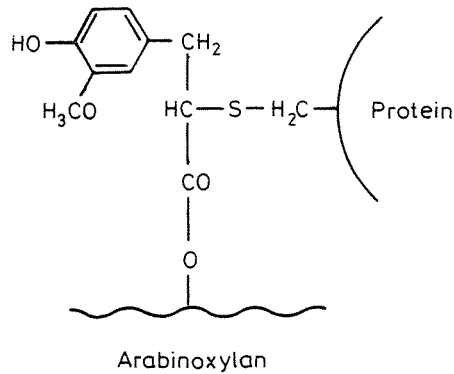

Arabinoxylan

Fig. 13. Suggested mechanism of oxidative gelation of pentosans (HOSENEY – FAUBION, 1981)

cules, hydrophilic interactions (hydrogen bonding) are possible between polar side chains or amino acid residues and the hydroxyl group of carbohydrates. A covalent bonding by O-glycoside or H-glycoside bonds is

characteristic of the majority of glycoproteins. Theoretically ionic bonding may not be excluded between oxidized derivatives of carbohydrates and ionic groups of the polypeptide chain. Fractionation and reconstitution studies have shown that starch tailings fraction, although contributing to water absorption and dough-fell, is not essential for optimum loaf volume (HOSENEY et al., 1971). KHAN – BUSHUK (1979) investigated the carbohydrate-containing glutenin fractions. A relatively significant amount of carbohydrates (6%) was detected in the high molecular weight fraction – separated by gel-filtration chromatography – of the reduced and alkylated glutenin. Sugar analysis (using paper chromatography) revealed that the carbohydrate moiety consists of arabinose, galactose, glucose, xylose and traces of mannose and glucose amines. SDS insoluble glycoprotein was isolated by GRAVELAND et al. (1982). Until now it was not possible to detect covalent bonding between glutenin proteins and carbohydrates. The exact knowledge of the type of interaction (hydrophilic or hydrogen bonds) needs further investigations.

The pentosans (water soluble and insoluble) of wheat flour were thoroughly investigated. A review paper was published by D'APPOLLONIA (1982). It was found that water insoluble pentosans improved the baking properties. The bread volume was increased, and, in addition, cell uniformity, crumb characteristics and elasticity of bread was improved. The nature of the interaction between the protein structure and pentosans is not yet knovn and needs further investigations. Oxidative gelation of wheat flour pentosans was observed (NEUKOM – MORKWALDER, 1978) and more recently the possible mechanism of oxidative gelation (involving proteins) was suggested by HOSENEY – FAUBION (1981). *Fig. 13* presents a possible way of covalent bonding between carbohydrates and protein side chains via phenolic compounds.

Experiments made recently in our laboratory have shown that hydrophilic, gel-forming carbohydrates (plant gums, pectic substances, etc.) added in small quantity (1 – 2%) improved the dough rheological properties and stability. Whether these effects are connected with carbohydrate–protein interaction or a discontinuous protein matrix 'mixed' with gelforming polysaccharides may also retain gas (carbon dioxide) is not clear at the present time. Theoretically an interaction of carboxyl groups of pectin and ionic groups of proteins is fully possible under the pH conditions prevailing in dough. The better understanding of these interactions will – we hope – open the possibility to involve some polysaccharides, too, in the dough conditioning.

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