Effect of whey peptides on surface activities of milk proteins

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RÉSUMÉ

Effet des peptides du lactosérum sur l’activité superficielle des protéines du lait

L’hydrolyse a été faite en utilisant la papaïne et les peptides qui en résultent ont été fractionnés par la chromatographie en phase inverse (C1, C8, et C18, attachés au grain de silicate). L’activité superficielle de la Kappa-caséine (K-CN) et de la β-lactoglobuline (β-LG) augmente lorsque ces protéines sont préalablement mélangées aux peptides. Cependant, quand les peptides sont disposés aux interfaces, ceux-ci deviennent hydrophobiques et répulsifs pour les protéines.

Mots clés
peptide, κ-caséine, β-lactoglobuline, protéine, papaïne.

SUMMARY

The overall functional properties of milk proteins may be influenced by whey peptides produced in food systems due to technical treatments. To study their effects, a hydrolysis was conducted using papain and the resulting peptides were fractionated by reverse phase chromatography (C1, C8 and C18 attached to silica beads). Results show that surfaces activities of κ-casein (Kappa-CN) and β-lactoglobulin (β-LG) increased when premixed with peptides. However, when the peptides were placed on the interface, the surface was hydrophobic and did not ease the spreading of proteins on the interface.

Keywords
peptide, κ-casein, β-lactoglobulin, protein, papain.

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1 – INTRODUCTION

The functionality of whey may be improved by modifying proteins. Their functionality is governed by the physico-chemical characteristics, interaction with other proteins, non protein components and environmental conditions of the food system.

Chemical-induced modifications are generally accomplished through acetylation, succinylation, imidation, esterification, chemical phosphorylation, thiolation, covalent attachment of amino-acid.

The effective hydrophobicity of certain globular proteins could conceivably be increased as well through exposure of a polar amino acid residues upon limited hydrolysis and subsequent unfolding of the polypeptide chain. Functional properties can vary depending upon the source, pre-treatment of whey, extent of the protein denaturation, and the presence of non protein components (MÖRR, 1976). Enzymatic modifications generally involve proteolytic hydrolysis of proteins to yield a mixture of peptides. The formation of bitter peptides upon hydrolysis of certain proteins is one of the problems sometimes encountered with the use of proteases (ALDER-NISSEN, 1976). This tendency to form bitter peptides is related to the content of hydrophobic amino acids. Caseins are examples of proteins with a propensity of developing bitter hydrolysates. New processing techniques have provided the dairy industry with additional possibilities for manufacturing dairy ingredients with specified dairy products. Some techniques such as ultrafiltration and enzymatic hydrolysis of the principal components of milk are extensively used. The demand for specialized dairy ingredients has been met by increased worldwide production.

Amphipathicity and micelle forming ability of proteins and peptides are properties being increasingly studied. There are four major casein components $\alpha_s1$-, $\alpha_s2$-, $\beta$- and $\kappa$-casein in proportions of 45, 12, 34 and 10% respectively depending on the genetic variant involved (RONALD, 1987) Casein has a high proline content uniformly distributed through the polypeptides chain, which limits the $\alpha$-helix or $\beta$-sheets formed. It has a very open structure compared with whey proteins. This open structure allows caseins to be easily accessible to various enzymes. This configuration accounts for the ability of caseins to diffuse readily at interfaces (DALGLEISH, 1987). The amphiphilic nature of caseins increases desired interactions with lipid-water or air-water interfaces playing thus an important role as a food emulsifier and stabilizer (DALGLEISH, 1987; RONALD, 1987). Proteins and amphipathic peptides once adsorbed at the interfaces orient themselves in such a manner that the hydrophobic segments of the protein molecule attach to the apolar groups of the lipid phase. This results in a reduction of the surface free energy that contributes to the protein stability. Kappa-casein is a more effective stabilizer than $\alpha_s1$-casein. Protein functionality can also be altered or extended by enzymatic action.

A study of whey protein hydrolysis with pepsin was reported by HAQUE (1992). Pepsin had an extremely detrimental effect on the emulsifying capacity. Extended periods of hydrolysis result in a drastic decrease of the foaming ability. Although there was an improvement in the specific foam volume upon limited hydrolysis, the stability was lowered compared to the control. The
amphipathicity of proteins therefore plays an important role in foams stability and viscosity.

This present study was conducted to show how whey peptides obtained by enzymatic hydrolysis could influence the surface activities of milk proteins especially κ-casein and β-lactoglobulin playing a major role in milk protein functionalities.

2 – MATERIALS

Imidazole/HCL and regular microscopic coverslips were purchased from Fischer scientific Co.

Whey protein concentrate was obtained from Mississipi State University (MSU), N, N dimethyl-3ammino-1 propane sulfonate (zw₈) and N-dodecyl-N N dimethyl-3ammono-1 propane sulfonate (zw₁₂) were obtained from Calbiochem, San Diego CA. Commercial grade 100% pure peanut oil used was purchased from Wal-mart. Methacryloxypropyltrimethoxysilane (Silane) from Sigma Chemical company, St Louis MO.

Kappa-casein (K-CN) was purified as mentioned in the methods with presep extraction columns: C₁ (trimethyl), C₈ (octyl), C₁₈ (octadecyl) and 45 µl liter were obtained from Fischer Fairlawn, NJ.

Acetonitrile and HPLC methanol were from J.T Baker Inc, Phillipsburg, NJ. Ammonium acetate was purchased from Aldrich Chem. Co. Inc Milwaukee WI. Papain was procured from Calbiochem CO, La Jolla, CA and then immobilized.

3 – METHODS

3.1 Preparation of model interface

Regular microscopic coverslips purchased from FISCHER Scientific CO. were silanised overnight by dipping them in methacryloxy-propyltrimethoxysilane and dried in vacuo.

Commercial grade peanut oil (10 µl) was applied to the edge of the glass slip and spread over a cm² area by gently smearing with the edge of an other glass slip. Depending on the experiment design, the interface was created by applying a drop of the dispersion directly to the oil smeared glass surface (design A) or pre-coated with 1 mg/ml methanolic dispersion of the peptides (design B described below) prior to use. The evaporation of was done during 10 min before the experiment was conducted. Precaution was taken not to touch the model interface during any stage of the experiment.
3.2 Preparation of crude peptides

Ultrafiltered whey (WPCX3) was hydrolysed using immobilized papain at pH 6 (acetate buffer) 37°C for 30 min. Papain was immobilized as described before (HAQUE, 1992). The hydrolysate was centrifuged at 32,500 g for 10 min and the supernatant was then filtered through a 45 μm filter.

The filtrate which consisted of a mixture of peptides has been termed the crude peptide in the text.

3.3 Hydrophobic peptides fractions

Peptides were fractionated from the crude peptides mixture by preparative chromatography using C1, C8 and C18 silicate beads. The hydrophobic peptides were bound to C1 beads. The eluted material was subsequently passed through the C8 and C18 columns. C18 were considered to the crude hydrophilic peptides fraction. The eluting buffer was acetonitrile/acetate buffer (pH 6) (8:1) followed by dioxane and finally double distilled water. Different peptides obtained from the different crude hydrolysate were dried in vacuo at 40°C and then re-dispersed to obtain 1 mg/ml solution in chromatography grade methanol. Eluted peptides from C1, C8, C18 columns were respectively called C1, C8 and C18 peptides. These were stored over a dessicant at 20°C until needed.

3.3.1 Experimental design A

A protein or protein + peptide dispersion was then applied to the silanised oily smeared. The glass was smeared with one drop of oil/cm².

a) one drop of double distilled water was applied on the oily smeared surface;

b) a drop of protein dispersion (K-CN or β-LG) was applied on the oily smeared surface instead of double distilled water.

3.3.2 Experimental design B

A Protein dispersion applied to silanised glass smeared with oil and pre-coated with peptides. One gram of peptides preparation was added to the oily surface at the center of the oil smear. This was allowed to spread and dry on the oily surface, giving the “pre-coated interface” referred to in the text:

a) one drop of glass distilled water was applied on the prepared surface;

b) one drop of protein dispersion (k-CN or β-LG) was applied onto an identical surface and the contact angle was then determined. The CA was the mean of at least three readings.

Precaution was taken to clean all utensils with chromatic acid and to use only glass distilled water.

3.3.3 Dynamic light scattering (DLS)

In order to understand the influence of the peptides on the surface activity of proteins, DLS of the proteins was carried out in the presence of model zwitterions (Z8, Z12 and Z16).
4 – RESULT AND DISCUSSION

The contact angle (CA) of proteins was recorded in the absence and in the presence of peptides. The β-LG on the uncoated interface and the crude peptide pre-coated interface gave a CA of 34° and 21.3°, respectively. The contact angles on these surfaces were significantly different at \( \alpha = 5\% \) (\( \text{Fc} = 116.76 \)) while the theoretical F value was 4.07 (\( \text{Ft} = 4.07 \)). Statistical analysis indicated that the interface pre-coated with the crude peptide gave means that were different. In the case of more amphipathic protein, K-CN, the CA on uncoated and “crude peptide” pre-coated interfaces was 34.3° and 8.3° respectively. Contrary to the CA of β-LG or the crude peptide itself, the CA of K-CN was dramatically reduced (four times) by the pre-coating of the interface with the crude peptide. As in the case of β-LG, the means of the two preparations were different when LSD was used. Once again the two observations were statistically different.

4.1 Fractionated Hydrophobic Peptides

When the fractionated hydrophobic peptides were used, the effects were completely different. When β-LG mixed with 4% (w/v) of \( C_1, C_8, C_{18} \) the CA values were 34, 35 and 36.5° respectively (figure 1). The value for β-LG alone was 38°. Interestingly the most hydrophobic peptide gave the lowest CA measurement reflecting a surface activity that was better than when the protein was alone. On the other hand, when the hydrophobic peptides were used to pre-coat the interface, CA were significantly higher for \( C_1, C_8, C_{18} \) the values were 64°, 53.7°, and 51° respectively (figure 1). These values were much higher than the CA of the β-LG by itself. The value was the highest for the most hydrophobic peptide fraction. This was contrary to the observations made with the crude peptide mixture where there was a decrease in the CA as a result of pre-coating interfaces.

![Figure 1](BL/C1 BL/C8 BL/C18 BL BL + C18 BL + C8 BL + C1)

Contact angle on a pre-treated glass surface (β-LG).
In the case of the less structured K-CN when C₁, C₈, C₁₈ peptides were mixed with it the contact angles were lowered than k-CN alone (35°) (figure 2). For C₁,C₈,C₁₈ the CA values were 34°, 33°, 32.2°, respectively. The lowest value of the most surface active state was obtained with C₁₈ (32°) (the least hydrophobic peptide fraction). This was contrary to the observation with β-LG where the best synergism was observed for C₁ which was the most hydrophobic peptide fraction. When the interface was pre-coated with the hydrophobic peptides, the trend was the same as for β-LG for C₁ C₈ C₁₈ the CA values were 49.7, 47 and 46 respectively (figure 2) The least hydrophobic peptides, C₁₈ had the least detrimental effect compared to the CA of the protein alone. The difference in the trend between the two proteins was observed when the hydrophobic peptides were in dispersion with the proteins and not when the peptides were located in the interface. This was conceivably due the structural effects. To explain this observation DLS experiments were conducted to observe the effect of model amphiphiles on the association properties of the proteins. (figure 3) zw₁₂ which is a hydrophobic zwitterionic amphiphile, markedly enhanced the tendency for β-LG; to associate. Based on the distribution percentage of β-LG aggregates as the concentration of zw₁₂ was increased from large (> 100nm) aggregates were formed. Such increase in the association tendency indicates an increase in the surface activity (HAQUE, 1992; HAQUE and KITO, 1983). In detailed study using various techniques, we observed that β-LG tends to be associated by small amphiphiles; casein was dissociated (HAQUE, 1992). Using model zwitterionic amphiphiles, it was shown by fluorometry that structural alterations were independent of the hydrophobicity of the amphiphile (HAQUE and MOZAFFAR, 1992).

In a related study, it was observed that the thermo-stability of K-CN and β-LG was affected by zwitterionic amphiphiles. While K-CN was destabilized as the hydrophobicity of the interacting amphiphile increased, β-LG became more stable. Recent observations using dynamic light scattering have clearly shown the powerful impact of the zwitterionic amphiphiles on protein-protein interactions (ABDUL-MANNAN et al., 1983; Dacre, 1993).

![Figure 2](image)

Contact angle on a pre-treated surface (κ-CN).
5 – CONCLUSION

The crude peptide mixture increased the surface activity of both β-LG and K-CN when it was located on the interface or co-dispersed with the proteins. On the other hand, fractionated hydrophobic peptides decreased surface activity of both the proteins when the pre-coated the interface. On the contrary, the co-dispersion of the hydrophobic peptides fractions with the proteins prior to the experiments resulted in increased surface activity indicating synergism. In this regard, the effect of the most hydrophobic peptides, C1 was significantly beneficial compared to the control. Data indicate that the effect of peptides may be beneficial or detrimental depending on the type of peptides. In general, hydrophobic peptides were detrimental when located on the interface irrespectively of the type of the protein used.
REFERENCES


