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Competitive Adsorption of Proteins from Total Hen Egg Yolk during Emulsification

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In this article, the competitive adsorption of egg yolk proteins at oil/water interfaces during emulsification is studied. By using two-dimensional polyacrylamide electrophoresis and mass spectrometry, it was possible to characterize and identify adsorbing and non-adsorbing protein species. The egg yolk contains proteins with a wide range of molecular weights and pI. Lipoproteins adsorbed selectively throughout the pH range investigated. It is suggested that selectivity is determined by the average hydrophobic and hydrophilic domain lengths in the protein sequences where long average hydrophobic domain lengths result in high affinity for the interface and thus strong preferential adsorption.

KEYWORDS: Egg yolk proteins; emulsion; competitive adsorption; oil−water interface; adsorption; lipoproteins

1. INTRODUCTION

Proteins play a large role in the formation and stabilization of food emulsions. Adsorbed proteins stabilize emulsions through electrostatic and steric stabilization. Steric stabilization is determined by the solubility of the protein in the aqueous phase, which in turn depends on protein charge. Thus, charge will determine both steric and electrostatic stabilization. In order to secure adsorption and be an efficient emulsifying agent, a protein should also possess the ability to unfold and spread at the interface (*1*). In the case of oil-in-water emulsions, the adsorption is mainly due to hydrophobic interaction between the protein and the interface, and thus, a high surface hydrophobicity of the protein is an important property (*2*) and may give rise to competitive adsorption. Although most proteins contain a sufficient mixture of hydrophobic and hydrophilic amino acid residues to be surface active and thus to adsorb at interfaces, kinetics and competitive strength may vary widely. Technical food proteins represent a mixture of protein species with different properties. Several authors have studied competitive adsorption between proteins at the oil/water interface (*3*−*6*), and Damodaran has written a good review covering competitive protein adsorption in general (*7*). Competitive adsorption can ultimately lead to only certain species present in a protein fraction being found at the interface. The protein that gives the highest reduction of the free energy of the system, that is, lowers the interfacial tension most efficiently, will dominate the interface at equilibrium. However, as reaching equilibrium can be a very slow process, other factors such as relative concentration and transport to the interface from the bulk solution will also influence the final composition of the interfacial layer. It has been shown that a less surface active protein can prevent the adsorption of a more surface active species (*8*). When adsorption has occurred, proteins can unfold and spread at the interface in order to optimize their configuration from a thermodynamical point of view (*8*−*10*). After this step, displacement of one protein by another may occur, although protein adsorption is to a large extent considered irreversible. Because the degree of unfolding depends on hydrophobicity/hydrophilicity of the interface, it follows that reversibility/irreversibility during competitive adsorption depends on the properties of the interface.

Egg yolk proteins are important in many disperse food systems as they are able to provide stabilizing effects at low pH. This is uncommon for proteins generally used in food emulsions, and hence, they are commonly used in the formulation of mayonnaise, sauces, and dressings. Although the yolk consists of other surface active components, such as phospholipids and cholesterol, the proteins have been shown to play a major role in emulsions, and the interaction between all the surface active constituents is likely to influence emulsification properties (*5*, *11*). However, it has been reported that the emulsifying activity of low-density lipoproteins (LDL) is due to the apoproteins rather than the polar lipids present in the lipoproteins (*12*, *13*).

The general characterization of egg yolk proteins has been proceeding since the early 1900s (*14*), and recently, there has been increasing activity in this field (*6*, *15*−*17*). Various mass

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spectrometric methods have facilitated the identification of individual proteins, and an example of this is a recent article by Tokarski et al. reporting the identification of egg yolk proteins extracted from renaissance paintings (*18*). However, to date there is little agreement on the molecular weight and identification of many of the proteins (*15*, *16*) obtained from gel chromatography of egg yolk or fractions thereof. The adsorption of egg yolk proteins in emulsions has also been studied. Mine studied the adsorption of egg yolk at the oil/water interface as a function of pH and oil volume fraction (*19*) and found that the constituents of the high-density lipoprotein fraction adsorbed preferentially. Mine also found that neither phosvitin, from granules, nor livetin, from plasma, adsorbed at the interface. It was also concluded that the low-density protein fraction was not a major contributor to emulsification. Le Denmat et al. studied the adsorption of egg yolk plasma and granules and found that plasma proteins were present at the interface both at pH 3 and pH 7 and at both 0.15 and 0.55 M NaCl (*11*). However, the composition of the adsorbed protein layer depended on the ionic strength and pH.

In a previous article (*6*) mass spectrometric techniques were used to identify competitive adsorption in the livetin fraction of egg yolk. It was reported that competitiveness depended on pH, although decomposition of proteins occurred at low pH (pH 4.0). Furthermore, surface loads in emulsions were determined using the serum depletion approach. The surface load values found were in the range of 1−2 mg/m^2, indicating monolayer adsorption, and were slightly higher, 2−4 mg/m^2, around the isoelectric point. In the previous study, the livetin fraction was chosen because of the high solubility of its protein species. While the total egg yolk is of a higher relevance for applications within disperse food systems, it is a substantially more complex system than the livetin fraction. This is because of the presence of lipids and components of poor or no solubility. Because of this, determination of surface loads as reported in the previous article (*6*) poses considerable difficulties.

The aim of this study is to investigate the identity, surface activity, and adsorption characteristics of egg yolk proteins in oil-in-water emulsions. Furthermore, any competitive adsorption of protein species will be investigated.

2. MATERIALS AND METHODS

Hen eggs were purchased from a local supermarket. The eggs were manually broken and the yolk and the white separated. The yolk was then carefully rolled on filter paper to remove remaining albumen according to the method of Le Denmat et al. (*11*). Buffers containing equal parts (10 mmol/L each) of lactic acid, acetic acid, imidazole, and TRIS to a total concentration of 40 mmol/L were prepared in range of pH 2.8−8.0 by the addition of either HCl or NaOH. The ionic strength was adjusted in the above buffers by the addition of NaCl. Oil-in-water emulsions were prepared with the appropriate buffer, 10% (v/v) medium chain triglyceride (MCT) oil (Miglyol 812, Sasol, Witten, Germany), and 1.2 g of yolk by mixing with an Ultra Turrax for 3 min followed by homogenization in a valve homogenizer at 15 MPa. The valve homogenizer used has been described in detail elsewhere (*20*). The ζ-potential of the emulsions was then measured with Zetasizer 4 (Malvern Instruments Ltd., Malvern, UK), and the area-weighted droplet diameter of the emulsion droplets was determined with light diffraction (Coulter LS 130, Beckman Coulter, High Wycombe, UK). Adsorption was determined through serum depletion, and the emulsions were separated in two steps by mild centrifugation in order to avoid coalescence until a clear subnatant was obtained. The first separation step was carried out at 3400*g* for 15 min and the second step at 10000*g* for 15 min. After the separation of the two phases, the tubes were vortexed in order to ensure that no sediment was formed. The non-adsorbing species in the emulsification experiments were determined with one-dimensional (1D) SDS−polyacrylamide electrophoresis and staining with Coomassie brilliant blue (*21*). In order to detect phosvitin some gels were stained with Coomassie brilliant blue containing Al-$(NO_3)_3$ as described by Hiramatsu et al.(*22*)

Gel spots were manually cut out, digested with modified porcine trypsin (20 ng/μL) (Promega, Madison, WI) according to the protocol supplied by Amersham Bioscience and spotted on MALDI target plates. MALDI-TOF spectra were acquired in a data-dependent mode on MALDI-HT (Waters, Sollentuna, Sweden). MALDI spectrum processing and database searches ($p < 0.05$) were performed using the PIUMS software (*23*, *24*). Sequence information was obtained by LC MS/MS spectra acquired on Qtof Ultima (Waters, Sollentuna, Sweden). MS/MS database searches were performed using Mascot Daemon (Matrix Science, www.matrixscience.com).

The scale of Kyte and Doolittle was used to assign hydropathy values to all amino acid residues in the peptide chains. A sliding average of 51 amino acid residues was used in order to identify hydrophobic loops in the protein sequence without confusing the interpretation by smaller hydrophobic patches that may be less likely to contribute to the adsorption affinity. The average domain length was determined and weighted with the integral of each domain, in order to reflect its magnitude of hydrophobicity or hydrophilicity according to

$$L = \frac{\sum_{j=1}^{m} l_j \sum_{i=1}^{n} (k_i + 0.4)}{\sum_{j=1}^{m} \sum_{i=1}^{n} (k_i + 0.4)} \quad (1)$$

where L is the average domain length, l_j is the domain length of the jth domain, k is the hydropathy score, n is the number of amino acid residues in the jth domain, and m is the total number of domains. The constant 0.4 is the hydropathy score of the glycine residue, which is considered to be neither hydrophobic nor hydrophilic.

Principal component analysis (PCA) was performed with The Unscrambler 9.0 (Camo ASA, Oslo, Norway).

3. RESULTS

The competitive adsorption of proteins from whole egg yolk during emulsification was studied. Emulsions at various pH were prepared with 10% (v/v) oil and 1.2% (w/v) egg yolk. The Sauter-mean droplet diameter and ζ-potential of the emulsions were determined after high-pressure homogenization. The emulsions were separated, and the non-adsorbing proteins were identified with mass spectrometry.

The emulsion surface area (A) depends on pH in the range investigated as shown in **Figure 1a**. At low pH, the surface area created during emulsification is high, reaching levels above 0.1 m^2/mL. The surface area then decreases strongly with increasing pH and reaches a minimum somewhere between pH 5 and 6. At higher pH, the surface area remains quite small (<0.05 m^2/mL). **Figure 1b** shows a plot of the ζ-potential as a function of pH. At low pH, the ζ-potential is highly positive, and it decreases strongly with increasing pH in a way similar to that of the surface area. The isoelectric point of the emulsion droplets occurs at a pH slightly above 5 which corresponds to the minimum in the surface area plot (**Figure 1a**), which is what could be expected because the stabilizing effect of the adsorbed protein layer would be at its minimum. At pH > 6, the ζ-potential shows a much weaker dependence on pH and seems to level off at value of about −15mV.

The results of the two-dimensional (2D) polyacrylamide gel electrophoresis are shown in **Figure 2**. Ten groups of spots were clearly identified, the multiple spots in each group being due to different degrees of phosphorylation. Spot 12 indicates the

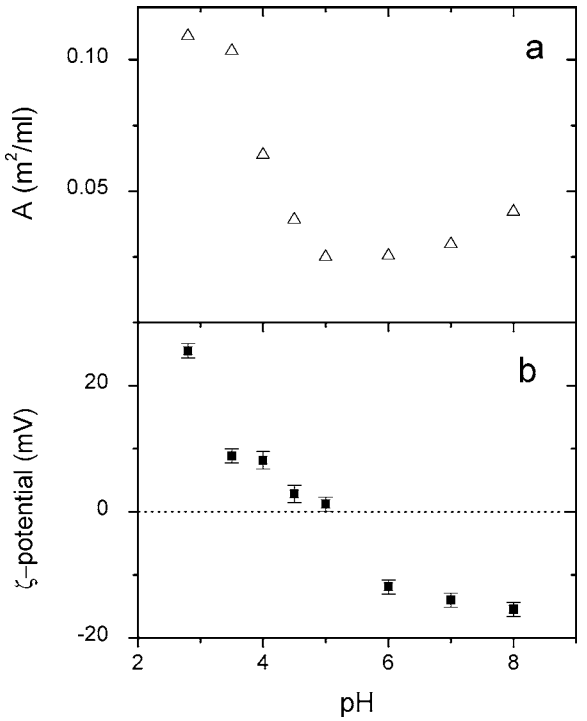

Figure 1. Results of the emulsification experiments shown as (**Figure 1a**) emulsion surface area (A) vs pH and (**Figure 1b**) the ζ-potential vs pH. The bars in **Figure 1b** show the standard deviation of the mean. The emulsion surface area was calculated from the Sauter mean droplet diameter (d_{32}).

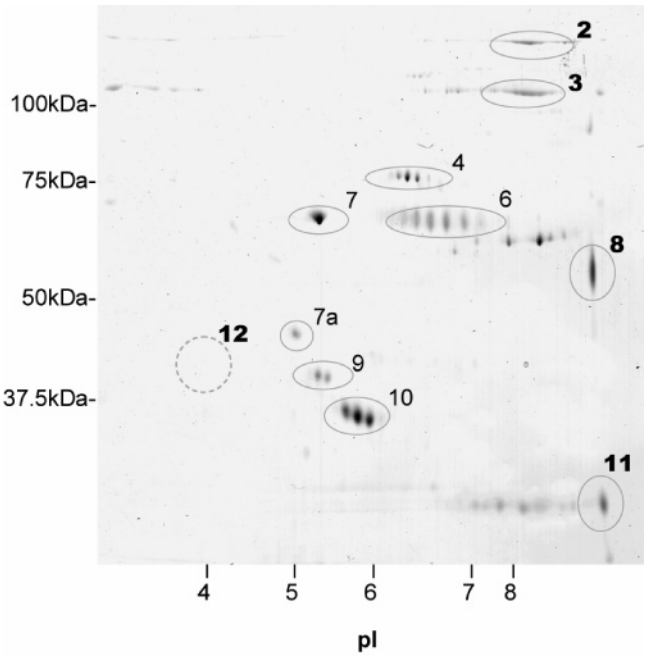

Figure 2. Two-dimensional polyacrylamide gel of whole egg yolk. The 10 most prominent protein bands, marked by rings, were identified, with the exception of spot 8, by mass spectrometry. The results are given in **Table 1**. The presence of several spots for many of the proteins is due to differential phosphorylation. Spots indicated with non-bold numbers belong to the livetin fraction.

location of phosvitin, which is only visible when the gel is stained in the presence of $Al(NO_3)_3$. The gel was stained in the absense of $Al(NO_3)_3$ to avoid interference with the staining of other protein spots. The molecular weight of the proteins ranges from about 28 kDa up to 240 kDa, and the pI varies from below 4 up to above 9 (**Table 1**). Samples were cut out from the gel for protein identification with MS; the results of the identification are included in **Table 1**.

A 1D gel stained with Coomassie Brilliant Blue of total egg yolk and of the unadsorbed protein fractions at different pH is shown in **Figure 3a**, and the lane profiles of the gel are shown in **Figure 3b** for comparison. The non-adsorbed protein fractions were obtained by gentle centrifugation of the emulsions. **Figure 3c** shows a 1D gel of the emulsification experiments which is stained in the presence of $Al(NO_3)_3$. This allows for the additional staining of phosvitin, which is being treated separately because, as mentioned above, $Al(NO_3)_3$ could influence the staining of individual protein bands. This in turn could influence the detection of competitive adsorption between species. From **Figure 3**, it can be seen that in particular some species, namely, the lipoproteins (marked by bold numbers), adsorb selectively throughout the pH range investigated. Phosvitin also adsorbs selectively except at pH 7 and 8 (**Figure 3c**), which could be due to high charge density of the protein at these pH values. Below pH 4 (**Figure 3**), some bands are present that are not observed in total egg yolk and must arise from the degradation due to destabilization of proteins at low pH. This makes determination of selectively adsorbing species difficult at these pH values. Significant degradation of some of the proteins was observed below pH 4, which made it difficult to determine the selective adsorption below this pH. A weak adsorption of Serum albumin (spot 7) occurs which is higher at the low pH. This corresponds well to previous findings (*6*). Ovotransferrin (spot 4) shows a weak tendency to adsorption at pH 4.0.

4. DISCUSSION

The results in this article show that the emulsifying capacity of egg yolk depends on pH, resulting in a larger emulsion interfacial area (smaller emulsion droplets) at lower pH. This is in contrast to results obtained by Mine, where larger interfacial areas were obtained at higher pH. (*19*) It is difficult to be certain of the origin this difference. Several experimental details differ, such as the emulsification method and the buffer substances employed. In the present work, the same buffering substances are present at all pH, and the ionic strength difference between buffers is compensated through the addition of NaCl. It is not clear from the information provided in the article by Mine (*19*) how such differences in ionic strength were handled, and hence, it is impossible to be conclusive on this issue.

The egg yolk lipoproteins and phosvitin adsorb selectively throughout the investigated pH range. The isoelectric point of the emulsion droplets occurs somewhere around pH 5.5 (**Figure 1b**), which is slightly surprising because the selectively adsorbing lipoproteins display pI > 8. However, phosvitin with its low pI (<4) (**Figure 2** and **Table 1**) and its inclusion in the adsorbed layer is likely to account for the relatively low isoelectric point of the emulsion droplets. In **Figure 1b**, it can also be observed that the slope of the ζ-potential plot decreases at higher pH, which can be explained from the composition of the adsorbed protein layer. At higher pH, the relative amount of phosvitin in the adsorbed layer diminishes. Hence, the ζ-potential does not decrease as would have been expected if the composition of the adsorbed layer were independent of pH.

In a previous article, we reported the selective adsorption of species from the livetin fraction of the egg yolk (*6*). In that study, it was observed that serum albumin (spot 7) and YGP 40 (spot 10) adsorbed, while the other proteins in that fraction largely remained in the continuous phase. The selective adsorption was attributed to long contiguous hydrophobic stretches in

Table 1. Twelve Dominant Protein Species in Egg Yolk As Determined by 1D and 2D SDS–PAGE and Subsequent Mass Spectrometry

spot	protein	observed MW (kDa)	observed pI	theoretical MW[a] (kDa)	theoretical pI[a]
1	vitellogenin-1	[b]	[c]	209	9.15
2	apoB, aa1–2017	240	8->9	224	8.68
3	lipovitellin-1	130	8–9	123	9.12
4	ovotransferrin (conalbumin)	80	6.5–7	75.8	6.69
5	vitellogenin, aa1–648	75	[c]	72.6	9.09
6	immunoglobulin G, heavy chain (γ-livetin)	65–70	6.5–8	(60–70)[d]	(6–7)[d]
7	serum albumin (α-livetin)	65	5–5.7	67.2	5.35
7a	truncated serum albumin (aa1–410)	45	5	47.0	5.45
8	apoB, aa2136–2554	55	>9	50.2	9.04
9	yolk plasma glycoprotein YGP42 (β-livetin)	40	5.3–5.8	31.4	5.88
10	yolk plasma glycoprotein YGP40 (β-livetin)	35	5.5–6.3	31.0	6.16
11	lipovitellin-2	28	>9	26.8	10.11
12	phosvitin	40	<4	31.4[e]	1.83[e]

[a] Not taking into account post-translational modifications, e.g., glycosylation or phosphorylation. Phosvitin (spot 12) is an exception. [b] The protein does not enter the polyacylamide separation gel. [c] The protein does not appear on the 2D gel. [d] The theoretical values for IgG are only approximate because of the inherent immunoglobulin heterogeneity. [e] Assuming 118 phosporylated serine residues (*43*).

the amino acid sequence in the adsorbing species, which were not present in the non-adsorbing species (*6*). In the present study, it can be seen that the protein species present in the livetin fraction remain largely un-adsorbed and thus play a less important role in emulsification with whole egg yolk, in agreement with results reported by Mine (*19*) and Le Denmat et al. (*11*). Both authors found that lipoproteins are the main contributors to the emulsifying capacity of egg yolk.

The selective adsorption observed in this article may be caused by several different factors. The lipoproteins adsorb selectively throughout the pH range, whereas proteins from the water soluble plasma fraction largely remain in solution. A major difference in solution behavior exists between these two groups of proteins because the lipoproteins are present in lipid aggregates (LDL or HDL). The lipids of these aggregates consist of apolar lipids such as triglycerides and polar lipids such as phospholipids and cholesterol. In the turbulent flow conditions during emulsification, convective mass transport can dominate over diffusion if the adsorbing molecules are large. If this is the case, large molecules will be transported to the interface more rapidly than small molecules (*25*−*28*). The lipoprotein aggregates are rather large (diameter of 17−60 nm for LDL and 7−20 nm for HDL (*29*)), and hence, a contribution from convective mass transport can be expected. However, in order to observe strong selectivity in adsorption, it is likely that larger sizes and a larger difference in size are required. Mizutani and Nakamura have shown that the emulsifying properties of LDL are to a large extent due to the lipoproteins rather than the phospholipids (*12*). This was further strengthened in another article by the same authors, where treatment of LDL with proteases inhibited emulsifying activity (*30*). It has been reported by Martinet el al. (*31*) that the LDL aggregate structure from egg yolk collapses upon impact with an interface, which results in a spreading of aggregate components at the interface. The collapse is likely to result in the dissolution of LDL triglycerides into emulsion droplets. Polar lipids could spread at the interface and be responsible for the sticking of the lipoproteins present. The predominant phospholipids of egg yolk are phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (99% of yolk phospholipids) (*32*), which are both zwitterionic. Malmsten et al. have studied the adsorption of a large hydrophobic protein (human apolipoprotein B) at interfacial PC layers with ellipsometry (*33*). The authors found very low adsorption, which increased when phosphatidic acid (PA) was included in the phospholipids layer. Hence, it is less likely that the protein−lipid interaction is the major contributor to selective adsorption. Rather, it is likely to be related to the true hydrophobicity of the protein species. The overall hydrophobicity/hydrophilicity (grand average amphiphilicity) values, as determined from the Kyte−Doolittle scoring system (*34*), are shown in **Table 2**. The grand average amphiphilicity does not differ greatly between the proteins; however, a weak trend does exist, the higher the score the stronger the adsorption, with some exceptions. The grand average amphiphilicty does not, however, explain the competitiveness stated in a previous article (*6*), showing that no general conclusions can be drawn from this value, which has also been discussed by Damodaran (*35*).

Another factor influencing selectivity could be the molecular weight of a protein because high molecular weight gives more potential sticking possibilities (per molecule) which should favor adsorption. The results show the adsorption of both high and low molecular weight species, and even though more of the high molecular weight species are preferentially adsorbed, the molecular weight does not have a strong overall influence on the competitiveness in the system. Some rather small proteins, such as lipovitellin-2, adsorb strongly, whereas some larger proteins such as ovotransferrin barely adsorb. Rather, selectivity could depend on the distribution of hydrophobic amino acid residues in the protein sequence because proteins can unfold and spread at the interface (*8*−*10*). Emulsifying activity is also known to be strongly related to the ability to unfold at the interface, and partially unfolded proteins often have higher emulsifying activities, which are related to a higher surface hydrophobicity and greater flexibility (*35*, *36*). The amount of spreading and the time scales depend on protein structure, solubility, and on the interface itself. Time scales of spreading have been reported to range between 10^{-2} and 10^{3} s (*37*, *38*). The importance of the hydrophobic block character for the adsorption of other surface active macromolecules such as triblock copolymers has been shown by several authors (*39*, *40*). The results of these studies show that a longer coherent block of monomers with a high affinity for the interface tends

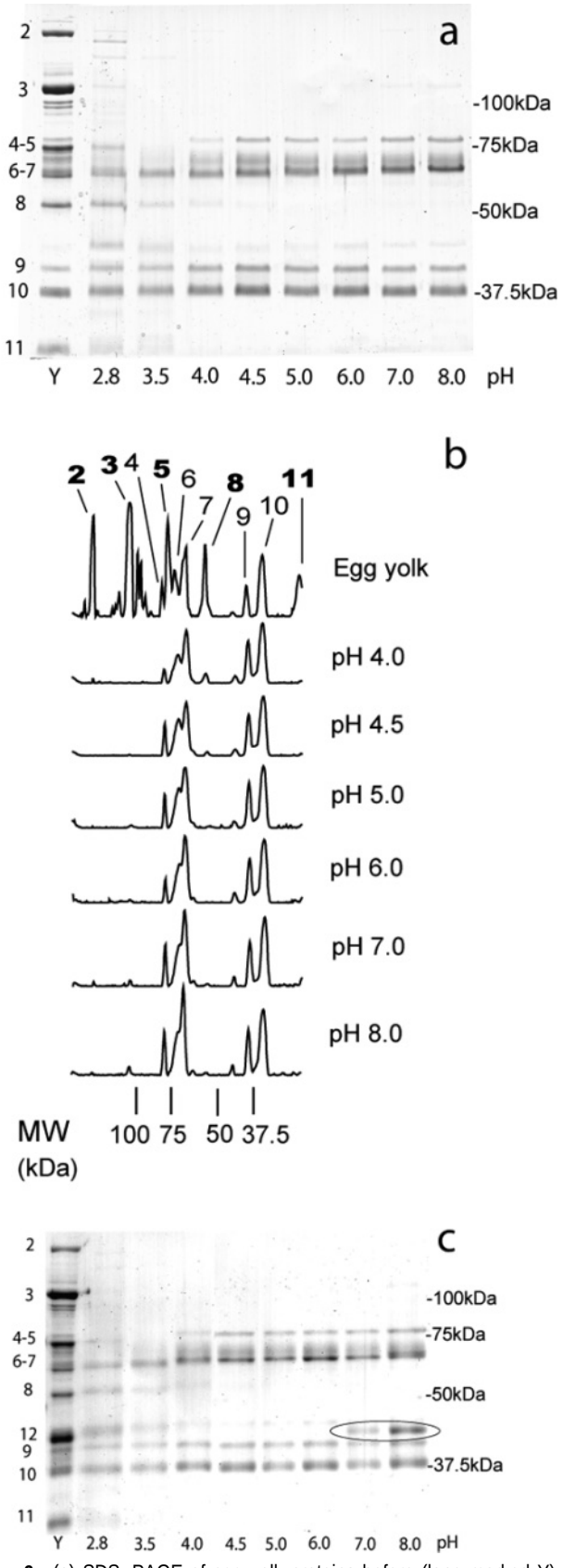

Figure 3. (**a**) SDS–PAGE of egg yolk proteins before (lane marked Y) and after emulsification at varying pH (noted below each lane) stained with Coomassie brillant blue. The numbering of the protein bands is the same as that in **Figure 2**. (**b**) Integrated lane profiles from **Figure 3a**. Spots indicated with non-bold numbers belong to the livetin fraction. (**c**) SDS–PAGE of egg yolk before and after emulsification at varying pH (noted below each lane) stained with Coomassie brillant blue and $Al(NO_3)_3$, which makes visible the protein band of phosvitin (marked with a ring).

to give greater surface activity and higher surface loads. Griffiths et al. have studied the role of copolymer architecture on adsorption at interfaces (*41*). The authors found that a cyclic triblock copolymer (in which the two hydrophilic blocks were covalently bound together) had adsorption behavior surprisingly similar to that of a linear triblock copolymer of the same composition. This is indeed an interesting result, which could to some extent be compared to the adsorption of proteins. Thus, the distribution of hydrophobic groups plays a large role in determining the amphiphilic character and surface activity of macromolecules, and we suggest that the results in this article also reflect this. In order to compare the amphiphilic character of the egg yolk proteins, the average hydrophobic and hydrophilic domain length was determined according to the scale suggested by Kyte and Doolittle (*34*). The calculation of averages is described in the Materials and Methods section of this article. The results are given in **Table 2**, together with the ratio between the hydrophobic domain length and hydrophilic domain length. From these results, it can be seen that long hydrophobic sequences tend to result in stronger adsorption. In order to achieve a clearer description of how the parameters correlate, a principal component analysis (PCA) was performed, and the results are displayed in **Figures 4** and **5**. **Figure 4** shows a rather clear grouping of the different proteins with less adsorbing proteins grouping to the left in the plot and more adsorbing species to the right. The loadings plot in **Figure 5** shows that adsorption is strongly related to the proteins being part of lipid aggregates and being promoted by a long average hydrophobic domain length. Hence, important factors determining adsorption efficiency are long hydrophobic stretches in the amino acid sequence and inclusion in lipid aggregates. The latter is possibly related to kinetic factors as outlined above. There is most likely a clear covariance between the two parameters.

Phosvitin is, because of its odd characteristics and unusual primary structure (123 of a total of 217 amino acid residues are serine, of which 118 are phosphorylated) (*42*, *43*), not included in the above discussion of selective adsorption. The molecule could to a large extent be considered as a highly charged polyelectrolyte (net charge of about −179 at pH 7) (*44*), and any treatment of its selective adsorption would, thus, have to take into account electrostatic considerations. The anomalous adsorption behavior of phosvitin has been investigated by Damodaran and Xu (*44*). At low ionic strength, phosvitin is complexed with the HDL fraction through calcium bridges (*13*), which further complicates the description of its adsorption behavior.

Other authors have also used utilized the Kyte−Doolittle approach (*34*) to describe the adsorption of proteins. Popineau et al. (*45*) studied the emulsifying properties of chymotryptic hydrolysates of wheat gliadins and found that selective adsorption of the more hydrophobic peptides occurred. Poon et al. reported on the emulsifying properties of apomyoglobin and a peptide (aa 1−55) derived thereof (*46*, *47*). The latter contained two major domains, one hydrophobic and one hydrophilic, and thus had a pronounced amphiphilic character to which the higher emulsifying efficiency was attributed. Thus, in the present article, it is suggested, with support from previous results (*6*) and the literature mentioned above, that the competitive adsorption of proteins from total egg yolk can be fairly well described from the primary structure of the proteins. Naturally, because of the complexity of proteins and their adsorption, a full explanation would require the use of additional parameters, for instance, the three-dimensional structure, structural flexibility, and the role of polar lipid−peptide interactions. However, the

Table 2. Properties of the Adsorbing and Non-adsorbing Protein Species

protein	spot	inclusion in lipid aggregates	sequence length	adsorption[a]	grand average amphiphilicity[b]	average domain length (*L*)[b]		domain ratio
						hydrophobic	hydrophilic	
apolipo B	2	yes	4204	strong	0.11	149	54	2.76
lipovitellin-1	3	yes	1096	strong	0.09	314	65	4.83
vitellogenin	5	yes	639	strong	0.18	147	32	4.59
apolipo B fragment	8	yes	487	strong	0.09	135	40	3.38
lipovitellin-2	11	yes	238	strong	0.10	58	42	1.38
serum albumin	7	no	597	medium	0	47	33	1.42
YGP 40	10	no	284	medium	0.16	122	45	2.71
ovotransferrin	4	no	686	weak	−0.04	42	59	0.71
YGP 42	9	no	285	weak	0.04	40	14	2.86

[a] The adsorption is qualitatively estimated from Figure 3. Species completely disappearing at all pH are denoted as strong adsorbates. The non-adsorbing or weakly adsorbing species in the present article are assigned as medium or weak adsorbates on the basis of their competitive strength in Nilsson et al. (*6*) [b] Hydrophobicity and hydrophilicity are determined from the scale suggested by Kyte and Doolittle (*34*).

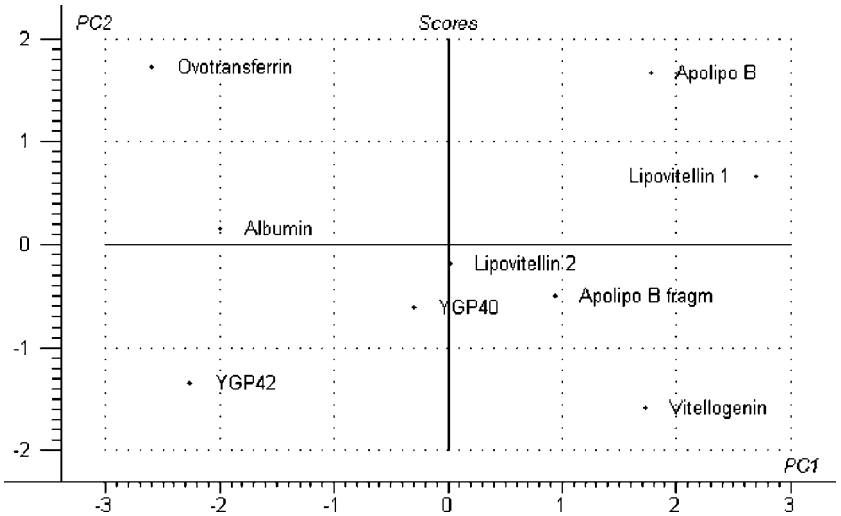

Figure 4. Score plot (PC2 vs PC1) from a principal component analysis showing the various protein species. Adsorbing species are preferentially distributed to the right, whereas the opposite applies for non-adsorbing species.

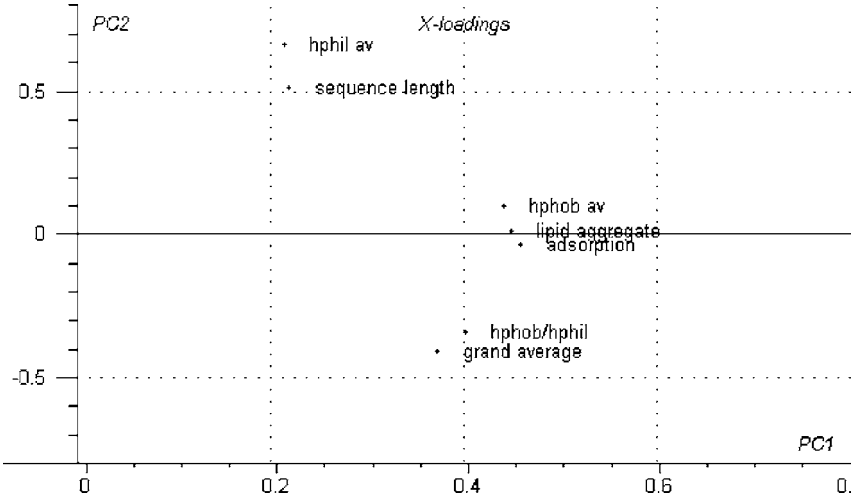

Figure 5. X-loadings plot (PC2 vs PC1) from a principal component analysis showing a strong correlation between adsorption and average hydrophobic domain length. The non-numeric variables, strong, medium, and weak adsorption, are given values of 3, 2, and 1, respectively, whereas inclusion in lipid aggregates was handled as 1 or 0.

approach used in the present article offers a powerful tool for the understanding of competitive adsorption from rather readily accessible quantities of proteins.

Thus, it is concluded that in this article the most abundant proteins of egg yolk were characterized with 2D gel electrophoresis and identified with mass spectrometry, and the adsorbing and non-adsorbing species at the oil/water interface during emulsification was determined. The use of these powerful techniques resulted in the possibility of understanding more about the adsorption behavior of proteins. Egg yolk constitutes

a mixture of proteins with a wide range of molecular weights and pI. Several of the species have pI values above 8. This could contribute to the good stabilizing properties of egg yolk at low pH because of the high relative charge density at low pH. The lipoproteins adsorbed selectively throughout the pH range investigated. We suggest that this selective adsorption correlates to the average hydrophobic domain length in the polypeptide chain and the ratio between hydrophobic/hydrophilic domain lengths. Long average hydrophobic domain length and short average hydrophilic domain length tend to result in stronger adsorption.

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