Effect of Denaturants on the Emulsifying Activity of Proteins

Simon Poon, Adrienne E. Clarke, and Carolyn J. Schultz*

Cooperative Research Centre for Bioproducts, School of Botany, University of Melbourne, Parkville, VIC 3010, Australia

The relationship between protein flexibility and emulsifying activity was investigated by disrupting disulfide bonds and/or noncovalent interactions of the protein. Oil-in-water emulsions using model proteins (apomyoglobin, β-casein, α-casein, lysozyme, bovine serum albumin, κ-casein, and β-lactoglobulin) were made in the presence of chemical denaturants (dithiothreitol and/or urea). In most cases, the presence of denaturants enhanced emulsifying activity. The effect was protein-specific and depended on the relative importance of disulfide bonds and noncovalent interactions in stabilizing the native conformation of each protein. Implications for the design of novel protein emulsifiers are discussed.

Keywords: *Protein emulsifier; emulsion; flexibility; protein adsorption; denaturation*

INTRODUCTION

The flexibility of a protein is an important feature affecting the emulsifying properties of proteins (Kato et al., 1985, 1986). In aqueous solution, the hydrophobic domains of a protein are generally buried in the interior of the molecule. To stabilize an emulsion, the hydrophobic domains of the protein should ideally be oriented toward the oil phase. The ease with which a protein is able to unfold (i.e., denature) to expose its hydrophobic domains therefore affects its emulsifying properties.

The three-dimensional structure of proteins can be stabilized by both covalent and noncovalent interactions. Covalent interactions consist of disulfide bonds, both intra- and intermolecular. Several approaches have been used to modify disulfide bonds and to test whether the resulting protein had enhanced emulsifying properties. Chemical reduction of the disulfide bonds of soy glycinin resulted in an increase in emulsifying and foaming activities (Kim and Kinsella, 1986, 1987). In these experiments the free thiol groups were alkylated to prevent the disulfide bonds from re-forming. The improvement in functionality may be attributed to increased conformational mobility, but the introduced alkyl groups may also have affected functionality by increasing hydrophobicity. Another approach is to eliminate cysteine residues using recombinant DNA technology. For example, the deletion of one cysteine residue of lysozyme resulted in enhanced emulsifying activity and emulsion stability (Kato et al., 1994). However, in another case, removal of the two disulfide bonds of soybean proglycinin (either or both) by substituting cysteine residues with other amino acids did not alter emulsifying properties (Utsumi et al., 1993). This may have been due to the remaining cysteine residues participating in intermolecular disulfide bond formation.

Protein flexibility is also affected by noncovalent interactions such as hydrogen bonding, van der Waal's forces, electrostatic links, and hydrophobic interactions. Heat denaturation, which disrupts these bonds, is one way of determining the importance of these forces in influencing the emulsifying activity of a protein. The emulsifying activity of several proteins is improved by heating, but the protein solubility is often lowered as a result of the exposure of previously buried hydrophobic domains (Voutsinas et al., 1983). Chemical denaturants, such as urea, have been used to study the interaction of denatured proteins at quiescent interfaces (Beverung et al., 1999). These studies are valuable in studying protein adsorption phenomena, but extrapolating the effects to food-based emulsion systems is problematic (Dalgleish, 1997). Emulsions in the food industry are formed using homogenizers, which impart a large amount of mechanical energy. Therefore, protein adsorption to the oil/water interface of food emulsions is not diffusion-controlled, as it is in the initial stages of protein−interface interactions in quiescent studies (Graham and Phillips, 1979).

The study presented here is an investigation of the effect that disrupting disulfide bonds and noncovalent interactions has on the ability of proteins to stabilize oil-in-water emulsions. To eliminate artifacts such as the introduction of thiol blocking groups and reduced solubility, emulsions were made (i.e., homogenized) in the presence of the denaturants, dithiothreitol (DTT) and/or urea. These chemicals are not approved as food additives, but they provide a simple means of determining the importance of protein flexibility on protein emulsifying activity, allowing hypotheses to be made regarding structure−function relationships. The results are discussed in the context of the molecular structure of each protein and, when relevant, their physiological functions.

MATERIALS AND METHODS

All proteins were purchased from Sigma (St. Louis, MO): lysozyme (L6876), β-lactoglobulin (L3908), myoglobin (M1882), α-casein (C6780), β-casein (C6905), κ-casein (C0406), and bovine serum albumin (BSA) (A4503). SDS−PAGE analysis showed that the purity of each sample was high (data not shown) and was consistent with the levels specified by Sigma. All proteins were used without further purification, with the exception of myoglobin. Apomyoglobin was obtained from

* Author to whom correspondence should be addressed (fax +61 3 9347 1071; e-mail c.schultz@botany.unimelb.edu.au).

10.1021/jf000179x CCC: $20.00
Published on Web 11/08/2000

Table 1. Effect of DTT and Urea on the Emulsifying Activity[a] of Model Proteins[b]

	emulsifying activity (mean droplet size, μm)			
protein	native	DTT[c]	urea[d]	DTT and urea[c,d]
apomyoglobin	7.6 ± 0.1	7.6 ± 0.5	6.3 ± 0.1	6.8 ± 0.2
β-casein	21 ± 1[e,f]	21 ± 1[e]	4.3 ± 0.3	4.0 ± 0.4
α-casein	7.6 ± 0.2	7.4 ± 0.3	4.7 ± 0.1	3.8 ± 0.4
lysozyme	51.5 ± 0.4	11.1 ± 0.2	34.6 ± 0.3	6.2 ± 0.1
BSA	7.8 ± 0.4	7.4 ± 0.1	21.7 ± 0.6	7.8 ± 0.3
β-lactoglobulin	30 ± 2	40 ± 1	9.0 ± 0.2	4.5 ± 0.0
κ-casein	9.2 ± 0.1	12 ± 1	5.1 ± 0.1	3.2 ± 0.0

[a] Emulsifying activity is indicated by the mean droplet size (d_{32}); confidence limits represent the standard error. [b] For conditions of emulsion test used, see Materials and Methods. [c] Concentration of DTT was 0.05 M. [d] Concentration of urea was 8 M. [e] Droplets flocculated. [f] An equal volume of 8 M urea added after emulsification reduced the extent of flocculation (visual examination) and decreased the droplet size to ~15 μm, suggesting bridging flocculation (Dickinson, 1997).

myoglobin by heme group extraction as described in Poon et al. (1999). Dithiothreitol (Bio-Rad, Hercules, CA) and urea (Sigma) solutions were made up to concentrations of 0.05 and 8 M, respectively.

Emulsions were prepared by homogenizing *d*-limonene (0.5 mL) (Bush Boake Allen, Dandenong, Australia) and 0.33 w/v % unbuffered protein solution [in either (1) 1.5 mL of deionized water (pH 7), (2) 0.05 M DTT, (3) 8 M urea, or (4) 0.05 M DTT and 8 M urea, for a final protein concentration of 0.25 w/v %] using an Ystral high-speed mixer (T1500, probe 3910F, Ystral). The limonene was weighted to a specific gravity of 0.9930 with sucrose acetyl isobutyrate (~36 vol %) in accordance with the manufacturer's instructions (Bush Boake Allen). The mean droplet sizes of the emulsions (by volume, d_{32}) were measured using a Coulter LS130 (Beckman Coulter Inc., Fullerton, CA) particle size analyzer and were determined at least 1 h after emulsion formation. Each emulsion was made at least twice. Creaming or settling/sedimentation was assessed qualitatively (i.e., by visual inspection), immediately preceding droplet size analysis. In this system, a more effective emulsifer is one that stabilizes a smaller droplet size. Droplets were also visually examined under a light microscope to check for droplet aggregation.

RESULTS

Emulsions were made by homogenizing limonene with aqueous protein solution alone or in the presence of 0.05 M DTT and/or 8 M urea. The emulsifying activity (droplet size stabilized) of each model protein and the effects of DTT and urea are shown in Table 1. The extent to which the oil droplets floated (creamed) or sedimented (settled) prior to droplet size analysis is shown in Table 2. Neither DTT nor urea had emulsifying activity in the absence of protein (data not shown).

Apomyoglobin. Native apomyoglobin was a good emulsifier, stabilizing a droplet size of 7.6 μm. DTT had little effect on the emulsifying activity of native apomyoglobin because emulsions formed with or without DTT had similar droplet sizes of 7.6 μm. The presence of urea resulted in a small improvement in emulsifying activity (6.3 μm droplets), as did the presence of both denaturants (6.8 μm droplets). Creaming occurred in emulsions when urea was present. The emulsions stabilized by native apomyoglobin and apomyoglobin/DTT neither creamed nor settled.

***β*-Casein.** Native β-casein was a poor emulsifier compared to the other caseins, producing large droplets (21 μm droplets) consisting of flocculated, smaller droplets. DTT did not improve its emulsifying activity.

Table 2. Effect of DTT and Urea on the Creaming Behavior[a] of Emulsions Stabilized by Model Proteins[b]

	creaming behavior			
protein	native	DTT[c]	urea[d]	DTT and urea[c,d]
apomyoglobin	–	–	creaming	creaming
β-casein	creaming[e]	creaming[e]	–	–
α-casein	settling	settling	–	–
lysozyme	settling	settling	creaming	creaming
BSA	–	–	severe creaming	creaming
β-lactoglobulin	creaming	creaming	slight creaming	–
κ-casein	–	–	–	–

[a] Assessed qualitatively ~1 h following emulsion formation ("–" indicates that neither creaming nor settling occurred). [b] For conditions of emulsion test used, see Materials and Methods. [c] Concentration of DTT was 0.05 M. [d] Concentration of urea was 8 M. [e] These emulsions had a foam layer.

There was a dramatic improvement in emulsifying activity when urea was present, either alone or in combination with DTT (4.3 and 4.0 μm droplets, respectively). Emulsions with no denaturants or with DTT exhibited unusual creaming behavior in that a foam layer was present on top of the emulsion, that is, on top of a creamed oil layer and aqueous subnatant. In contrast, emulsions containing urea did not cream, settle, or foam, nor were the droplets flocculated.

α-Casein. α-Casein was the most active of the three casein emulsifiers tested, stabilizing a droplet size of 7.6 μm. The presence of DTT had little effect. However, urea or the combination of urea and DTT improved the emulsifying activity (4.7 and 3.8 μm droplets, respectively). Emulsions stabilized by native α-casein and α-casein/DTT settled. Neither settling nor creaming occurred with emulsions containing urea.

Lysozyme. Native lysozyme was a poor emulsifier (50 μm droplets). The presence of DTT dramatically improved the emulsifying activity (11.1 μm droplets). The droplet size was further reduced to 6.2 μm when both urea and DTT were present. The presence of urea alone resulted in only a slight improvement in emulsifying activity (34.6 μm droplets). The emulsions that contained urea creamed. In contrast, emulsions stabilized by native lysozyme and lysozyme/DTT settled.

BSA. BSA had an emulsifying activity similar to that of apomyoglobin (7.8 μm droplets). Both DTT and the combination of DTT and urea had little effect on the emulsifying activity of BSA. However, the presence of urea alone decreased emulsifying activity (21.7 μm droplets), and the emulsion creamed.

***β*-Lactoglobulin.** Like lysozyme, native β-lactoglobulin had poor emulsifying activity (30 μm droplets). However, unlike lysozyme, DTT did not decrease the droplet size of β-lactoglobulin-stabilized emulsions. Both of these emulsions creamed. The presence of urea improved the emulsifying activity of β-lactoglobulin (9.0 μm droplets). This emulsion exhibited only slight creaming. The presence of both urea and DTT resulted in a further improvement in the droplet size (4.5 μm droplets), with neither creaming nor settling.

***κ*-Casein.** Native κ-casein was an effective emulsifier (9.2 μm droplets). The presence of DTT slightly decreased the emulsifying activity (12.0 μm droplets). Urea increased the emulsifying activity (5.1 μm droplets), whereas the combination of urea and DTT resulted in a further improvement (3.2 μm droplets). The emulsions stabilized by κ-casein neither creamed nor settled.

Table 3. Disulfide Bonding of Model Proteins and Other Features That May Influence the Effect of DTT on Emulsifying Activity

protein	mass (kDa)	no. of amino acids	no. of −S−S−	amino acid residues spanned by disulfide bonds[a]	dimers, multimers, or self-association[b]	other features	effect of DTT on emulsifying activity
apomyoglobin	16.9	153	0				none
β-casein	24.0	209	0		self-assoc		none
α-casein[c]							
α_{s1}	23.6	199	0		self-assoc		none
α_{s2}	25.2	207	2	36−40 (intermolecular)	dimers (cov)		
lysozyme	14.4	129	4	**6−127**, **30−115**, 64−80, 76−94			enhanced
BSA	66.3	582	17	53−62, 75−91, 90−101, 123−167, 166−175, 198−244, 243−251, 263−277, 276−287, 314−359, 358−367, 390−436, 435−446, 459−475, 474−485, 512−557, 556−565		lipid binding and transport	marginal positive effect
κ-casein	19.0	169	2	11−11, 11−88, 88−88 (all intermolecular)	multi (cov)		diminished
β-lactoglobulin	18.3	162	2	**65−160**, 106−119 or −121	dimers (nc)	nonpolar ligand site	diminished

[a] Boldface entries indicate disulfide bonds link distant parts of the peptide chain, relative to the size of the chain itself. [b] State of native aqueous protein solution prior to emulsification (self-assoc, self-association; multi, multimers; cov, covalently bonded; nc, noncovalently bonded. [c] Ratio of α_{s1}:α_{s2} ∼ 4:1.

DISCUSSION

Emulsifying activity was evaluated by the mean droplet diameter of an emulsion of limonene stabilized by proteins in the presence or absence of denaturants. We used a small-scale emulsion test based on that used in the beverage industry to make citrus flavor emulsions for soft drinks (Ray et al., 1988). This test enables small quantities of material to be assessed for emulsifying activity. A range of droplet sizes is obtained for different emulsifiers, allowing small differences in emulsifying activity to be detected (Poon et al., 1999). In this study, the droplet sizes were larger than the 1 μm (or less) required for industrial use in soft drinks. During the homogenization step of emulsion preparation, protein molecules adsorb to the oil/water interface, thereby providing a barrier against droplet coalescence. The effectiveness of a protein emulsifier depends on both its ability to reduce the droplet size during homogenization and its subsequent ability to stabilize the droplet size after homogenization. In this study, we have focused on the first of these factors as the influence of denaturants is likely to have greatest effect during the initial adsorption to the oil phase.

Effectiveness of Each Protein as an Emulsifier. In the absence of denaturants, the model proteins had very different activities as emulsifiers (Table 1). For example, lysozyme was very poor (stabilizing droplets of 51.5 μm), whereas apomyoglobin was relatively effective (stabilizing droplets of 7.6 μm). The explanation for the foaming observed with β-casein is uncertain. However, studies have shown that β-casein is highly surface active at the air/water interface (Dickinson, 1998), and it may be this property that promotes foaming. The relationship between protein structure and emulsifying activity depends on a combination of factors such as molecular flexibility, molecular size, surface hydrophobicity, net charge, and amino acid composition (Turgeon et al., 1992).

Effect of Disulfide Bond Reduction on the Emulsifying Activity of Proteins. As expected, the emulsifying activities of the proteins lacking disulfide bonds (apomyoglobin, β-casein, and, to a large extent, α-casein) were unaffected by DTT (Table 1). In proteins containing disulfide bonds, reduction of these bonds by DTT was expected to increase emulsifying activity by allowing the protein to adopt conformations that expose hydrophobic domains normally buried in the native protein.

This was the case for lysozyme, a small globular protein with four disulfide bonds. In the presence of DTT, the emulsifying activity of lysozyme was markedly enhanced. The location of the disulfide bonds may be an important factor for this enhancement. Two of the disulfide bonds span long stretches of the 129 amino acid polypeptide chain: amino acid residues 6−127 and residues 30−115 (Table 3). Hence, these two disulfide bonds are likely to play a major role in maintaining the folded, three-dimensional structure of lysozyme. This interpretation is consistent with the small improvement in emulsifying activity obtained when disulfide bond 76−94 of lysozyme was genetically deleted (Kato et al., 1994). A larger improvement might be expected if disulfide bonds 6−127 and 30−115 of lysozyme were deleted.

In contrast to lysozyme, the other proteins with disulfide bonds (BSA, β-lactoglobulin, and κ-casein) that were tested did not behave as predicted. This anomalous behavior is likely to be due to specific characteristics of these proteins. Each of the key features of these proteins (Table 3) will be discussed in relation to their response to DTT.

BSA is a large molecule (66.3 kDa) with 17 disulfide bonds. Dynamic interfacial tension measurements at a heptane/aqueous buffer interface suggest that native BSA is highly surface active (Beverung et al., 1999). This is consistent with the high emulsifying activity of native BSA observed in this study. The presence of DTT only marginally improved the emulsifying activity of BSA over that of the native protein. It is likely that the native conformation of BSA is conducive to emulsification given that BSA functions as a lipid binding and transporting protein in vivo (Peters, 1985). An alternate possibility for the lack of improvement in emulsifying activity is that the disulfide bonds are not fully accessible to DTT. Indeed, these bonds are not accessible to thioglycolic acid, another reducing agent, in the pH range 5−7 (Katchalski et al., 1957).

Surprisingly, DTT had a negative effect on the emulsifying activities of β-lactoglobulin and κ-casein. In the case of β-lactoglobulin (two disulfide bonds), the presence of DTT increased the droplet size from 30 to

40 μm. One possible explanation relates to the native structure of β-lactoglobulin, which incorporates a nonpolar ligand binding site. This binding site consists of a pocket lined with hydrophobic amino acid side chains and is believed to sequester a small, nonpolar molecule, retinol, almost entirely from the external aqueous environment (Papiz et al., 1986). The hydrophobic pocket includes two cysteine residues, one of which participates in disulfide bond formation (position 119 or 121). Cleavage of the disulfide bonds may lead to the collapse of this hydrophobic pocket, resulting in stronger intramolecular interactions and a consequent negative effect on the emulsifying activity of β-lactoglobulin in the presence of DTT.

κ-Casein has a physiological role in stabilizing casein micelle formation and preventing calcium precipitation. The two cysteine residues can participate in intermolecular disulfide bonding, resulting in structures ranging from a monomer to multimeric structures larger than a decamer (Rasmussen et al., 1992). The detrimental effect of DTT on the emulsifying activity suggests that the multimeric structure is important for emulsification. The native monomer may have few hydrophobic domains that can be oriented efficiently at the oil/water interface, and it is only when multiple κ-casein molecules are linked that a significant hydrophobic domain is created. This interpretation is consistent with the hypothesis that the multimeric structure of bovine κ-casein facilitates coverage of the casein micelle surface, thereby stabilizing the micelle structure (Rasmussen et al., 1992).

In summary, the reduction of disulfide bonds by DTT did not result in an enhancement of emulsifying activity in all proteins tested. The physiological roles of the protein, the length of polypeptide spanned by disulfide bonds, and their accessibility are factors that may influence the effect.

Effect of Disruption of Noncovalent Interactions on the Emulsifying Activity of Proteins. Urea enhances protein flexibility by disrupting noncovalent interactions that theoretically should increase emulsifying activity. This was the case for all proteins except BSA (Table 1). For some proteins (apomyoglobin, lysozyme, and κ-casein) the enhancement in emulsifying activity in the presence of urea was probably due to disruption of intramolecular noncovalent bonds (i.e., secondary and tertiary structure), whereas for others (α- and β-casein and β-lactoglobulin) disruption of intermolecular noncovalent bonds (i.e., quaternary structure) may also be involved.

The results also provide information about the likely importance of secondary, tertiary, and quaternary structure in maintaining the native structure of each protein. For example, the enhancement of the emulsifying activity of κ-casein by urea suggests that noncovalent interactions are important in stabilizing the three-dimensional structure of this protein. In this situation, the multimeric structure of κ-casein would not likely be affected, but the orientation of the hydrophobic domains may be altered to allow greater freedom to adsorb to oil droplets.

The improvement in emulsifying activity of lysozyme in the presence of urea was less than that in the presence of DTT (Table 1). Hence, disulfide bonds may play a more important role in stabilizing the three-dimensional structure of lysozyme than noncovalent interactions.

The improvement in emulsifying activity of α- and β-casein produced by urea is likely to be due to disruption of self-association (Schmidt et al., 1982). This would allow hydrophobic domains to adsorb to oil droplets rather than to other casein molecules. Furthermore, the disruption of the self-associated β-casein by urea would make monomers available for adsorption to the oil phase, reducing bridging flocculation (Table 1).

In β-lactoglobulin, noncovalent interactions play a role in stabilizing tertiary structure as well as in the formation of noncovalently linked dimers (Swaisgood, 1982). Disruption of these interactions by urea is likely to have resulted in the improvement in emulsifying activity measured.

The emulsifying activity of apomyoglobin was marginally improved in the presence of urea. The minimal change may be due to similarity in the surface hydrophobicity of the native and denatured protein. This is consistent with previous work which suggests that apomyoglobin adsorbs to the oil phase in a conformation similar to that of the native protein (Poon et al., 1999).

The detrimental effect of urea on the emulsifying activity of BSA was unexpected given that the emulsifying activity of every other protein in this study was improved in the presence of urea. Previous experiments have also found that urea-denatured BSA had low emulsifying activity compared to that of native BSA (Waniska et al., 1981). It was suggested that this was due to native BSA forming a stronger, more cohesive interfacial film due to the presence of more tertiary structure. A similar effect was found with ovalbumin, where a urea-free solution exhibited a faster initial increase in surface pressure than the protein in the presence of 6 or 9 M urea (Beverung et al., 1999). Thus, in this case and in the case of BSA, urea reduced the driving force for adsorption of hydrophobic groups to the oil phase. The large size of denatured BSA (and of ovalbumin, molecular mass = 42 kDa) may be responsible for the molecule's adsorbing less efficiently to the oil/water interface. Urea may also disrupt the noncovalent tertiary folding that forms lipid binding sites in the native molecule (Peters, 1985).

The emulsifying activity of urea-denatured BSA was enhanced when DTT was present. One interpretation is that denaturation by urea provided access for DTT to reduce otherwise solvent-inaccessible disulfide bonds. The exposed hydrophobic domains could then adsorb to the oil phase. The net result was an emulsifying activity similar to that of native BSA but significantly better than that of BSA in the presence of urea alone.

Effect of Reduction and Denaturation on the Emulsifying Activity of Proteins. The combined effect of disulfide bond reduction by DTT and the disruption of noncovalent interactions by urea results in proteins that lack ordered structure. The presence of urea would theoretically make all disulfide bonds accessible to DTT and therefore improve the emulsifying activity compared to the situation in which DTT was used alone.

Even under fully reduced and denatured conditions, there were differences in emulsifying activity between proteins (3.2−7.8 μm). This suggests that protein flexibility is not the only factor influencing emulsifying activity. The range of droplet sizes obtained under these conditions may be due to differences in amino acid composition and/or sequence. As hydrophobic amino acids are responsible for adsorption to the oil phase,

average amino acid hydrophobicity may be an important factor in emulsifying activity. However, no correlation was found between droplet size and average amino acid hydrophobicity of the test proteins (data not shown). Thus, it is likely that amino acid distribution plays a crucial role in determining emulsifying activity in proteins.

Creaming Behavior. Hydrodynamically, emulsion droplets can be viewed as hard spheres, and Stokes's law has been applied to describe their behavior (Pinfield et al., 1994). This states that creaming is proportional to the square of the droplet diameter and the density difference between the two phases and is inversely proportional to the viscosity of the continuous phase.

In this study (Table 2), two factors appear to influence creaming behavior. First, the presence of 8 M urea promoted creaming, probably due to its higher density (~1.15 g/mL compared with 0.993 g/mL for the weighted oil). Second, a droplet size greater than ~20 μm was associated with creaming. The sedimentation of emulsions stabilized by lysozyme and α-casein in their native form and in the presence of DTT is more difficult to explain. Presumably, the adsorption of these proteins to the oil droplets under these conditions increased the density of the dispersed phase compared with that of the aqueous phase (Hill, 1996). However, the mechanism by which this occurred is not known.

Approaches for Improving the Emulsifying Activity of Proteins. An important consideration for protein emulsifiers highlighted in this study is the quaternary structure of proteins. Self-association by hydrophobic interactions was implied as a factor that limited the emulsifying activity of α- and β-casein. The relatively large size of the hydrophobic domains of these proteins is likely to be responsible for such intermolecular interactions. It is possible that the hydrophobicity of these domains in monomers is more than sufficient for good emulsifying properties. A reduction in hydrophobicity which reduces the tendency for intermolecular interactions may be possible without adversely affecting emulsifying activity. Alternatively, reducing the size of the hydrophobic domains (e.g., by either genetic engineering or enzymatic or chemical hydrolysis) may have a similar result. Thus, further work is required to determine the balance of hydrophobicity required for efficient emulsifying activity while minimizing self-association. Such a balance exists for low molecular weight, surfactant-type emulsifiers for which hydrophile−lipophile balance (HLB) values are used for selecting an appropriate emulsifier (St. Angelo, 1989). The complexity of protein structure has so far prevented a similar scale to be developed for proteins.

Another strategy for the improvement of emulsifying activity by genetic engineering would be the introduction of proline residues, which disrupt secondary structure. This may increase flexibility, promoting the disordered conformations of α- and β-casein, two proteins rich in proline (Swaisgood, 1982).

The efficacy of some proteins as emulsifiers in food systems has similarities with their physiological roles. For example, the binding of nonpolar molecules (e.g., retinol and lipids by β-lactoglobulin and BSA, respectively) is a physiological function that in some respects is similar to the emulsifying requirements of adsorption to an oil phase. The key difference lies in the binding of a single or small number of molecules physiologically, compared with the binding to an interface in the case of an emulsion. For example, under native conditions, β-lactoglobulin is designed to bind a single molecule of retinol. The tertiary structure required for this function is not suitable for adsorption to an interface (nor can the native structure rearrange to make it so), as reflected in the large droplet size stabilized (30 μm). However, reduction and denaturation of the molecule allow the domains that are responsible for retinol binding to interact readily with an oil/water interface. The small droplet size stabilized by denatured and reduced β-lactoglobulin (4.5 μm droplets) demonstrates that these domains are highly effective in an emulsifying role.

This suggests a strategy for obtaining active protein emulsifiers by using appropriate, shorter stretches of the protein, produced either through a genetic engineering approach or through selective enzymatic or chemical hydrolysis. In particular, the targeting of those domains directly involved in nonpolar ligand binding may yield molecules with higher emulsifying activity than the parent protein. For example, tryptic hydrolysis was used to obtain active emulsifying peptides from the β-barrel domain of β-lactoglobulin (Huang et al., 1996). This is the domain responsible for retinol binding. Peptide emulsifiers could also be obtained by expressing genes encoding the relevant domains considered to be important for nonpolar ligand binding. It could also be useful to investigate other proteins with similar physiological roles, such as apolipoproteins, for their emulsifying properties and to test whether their binding sequences could also yield good emulsifiers.

The experiments described here provide a rapid method for testing proteins for their potential as emulsifiers. Such experiments could be a prelude to further work using techniques such as genetic engineering or protein hydrolysis to produce highly effective protein emulsifiers.

ACKNOWLEDGMENT

We thank Justin Cooper-White, Department of Chemical Engineering, University of Melbourne, for helpful comments during the preparation of the manuscript.

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Received for review February 11, 2000. Revised manuscript received September 5, 2000. Accepted September 6, 2000. This research was supported by the Australian Food Industry Science Centre through a postgraduate scholarship to S.P. and by the Australian Federal Government through the Cooperative Research Centre for Bioproducts.

JF000179X