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RESEARCH NOTE

Two amphiphilic, synthetic peptides display strong emulsification properties

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Two amphiphilic peptides with the following sequences were designed and synthesised: peptide 1, TFLQDLKEKVQQLTEALK; peptide 2, TVSQLQEYWT-TLLSQIKTLLQQIKTS. The emulsification properties of these peptides were tested in an oil–water two-phase system and were found to be significantly greater than those of mellitin. Circular dichroism measurements were used to characterise the secondary structure of the peptides. In aqueous buffer, they adopted *c.* 50% beta sheet conformation.

INTRODUCTION

One of the major contributions of proteins, such as caseins and albumin, to functionality in food systems is their emulsification (Macritchie, 1978). Studies on the role of proteins in this process have, of necessity, often been empirical in the past and have concentrated on heterogeneous mixtures of proteins and peptides in an attempt to mimic the conditions obtaining in a real food system. It has therefore been difficult to correlate the structure of the peptides/proteins with their properties as emulsifiers although, in a previous study, Shimizu *et al.* (1984) demonstrated that peptide 1-23 of αS1-casein alone possesses considerable emulsification properties.

From our knowledge of other surface-active proteins such as lipoproteins and toxins, one of the main structural features which we would expect to contribute to emulsification is amphiphilicity. Frequently, surface-active proteins possess amphiphilic alpha-helix components. Developments in the area of computer modelling of peptides (Olsen & Goodsell, 1992) and in their synthesis by solid phase methods (Merrifield, 1963) have now made it feasible to design and prepare peptides containing defined structural features (Taylor & Kaiser, 1987; Degrado, 1988). Studies with such peptides ought to make it easier to correlate peptide conformation with emulsification properties.

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In the present paper, we report the synthesis and characterisation of two peptide sequences which possess significant emulsification properties in an oil–water system.

MATERIALS AND METHODS

Peptides 1 (TFLQDLKEKVQQTEALK) and 2 (TVSQLQEYWTTLLSQIKTLLQQIKTS) were modelled using INSIGHT II on an Iris 40 personal computer from Silicon Graphics. These sequences were found to optimise amphiphilicity. Peptides were then synthesised on an Applied Biosystems 431A solid phase peptide synthesiser, using either Fmoc (peptide 1) or Fastmoc (peptide 2) chemistries (Fields & Noble, 1990).

The peptides were characterised by N-terminal microsequencing (National Food Biotechnology Centre, University College Cork, Republic of Ireland), reversed phase high-performance liquid chromatography (HPLC) and thermal desorption mass spectrometry (Queens Medical Centre, Nottingham, UK).

Emulsification was assessed in 10 ml 1:1 mixtures of rapeseed oil (containing 5% Sudan III dye)–20 mM sodium acetate/acetic acid buffer, pH 4.8. Preweighed quantities of peptide were added and emulsions were formed by homogenisation in a Polytron homogeniser (4 min, speed 4). The time required for phase separation at 6°C was noted and the time taken for separation of a blank emulsion (usually 2 h) was taken as zero time. Emulsions for circular dichroism (CD)

Table 1. Circular dichroism spectroscopy of model peptides 1 and 2 in a variety of solvents

	Alpha-helix	Beta-sheet	Random coil
Model peptide 1			
20 mM Na acetate (buffer, pH 4·8)	5 (0)	51	44
20 mM Na acetate (buffer, pH 4·8, 50% TFE)	45 (49)	55	0
20 mM Na acetate (buffer : rapeseed oil)	— (27)	—	—
Model peptide 2			
20 mM Na acetate (buffer, pH 4·8)	13 (14)	53	33
20 mM Na acetate (buffer, pH 4·8/50% TFE)	33 (32)	58	9
20 mM Na acetate (buffer : rapeseed oil)	— (11)	—	—

In the case of the oil-water emulsion, spectra were recorded only at wavelengths ≥210 nm and were averaged over at least three measurements. The data were fitted with the method of Siegel *et al.* (1980), which allows estimation of alpha-helix alone. The result from this latter procedure is shown in parentheses. Peptide concentrations were 0·38 to 0·5 mg/ml.

measurements were generated by sonication of the above oil–water system (4 × 10 s, 9μm amplitude) before addition of the test peptide (0.5 mg/ml final concentration). In these experiments, Sudan III dye was omitted.

CD spectra were recorded on a JASCO J-600 spectropolarimeter (20°C, cell pathlength 0.02 cm) in solvents detailed in Table 1. For most measurements, the CONTIN method of Provencher and Glöckner (1981) was used to estimate percentages of secondary structure. However, for measurements in oil–water emulsions, the method of Siegel *et al.* (1980) was used to fit data at wavelengths ≥ 210 nm, yielding measurements only of percentage alpha-helix.

RESULTS AND DISCUSSION

The successful synthesis of peptides 1 and 2 was confirmed by reversed-phase HPLC, mass spectrometry and N-terminal microsequencing. The emulsification properties of peptides 1, 2 and mellitin in the oil–water assay are shown in Fig. 1. Both of the synthetic peptides were found to be better emulsifiers in this system than mellitin and emulsification by peptide 2 was especially strong, being greater than the values we obtain in this system with known emulsifiers such as lecithin and gum guar. From this we conclude that amphiphilicity of peptides is indeed a major structural requirement for their use as emulsifiers.

The CD spectrometry of peptides 1 and 2 is shown in Table 1. This indicates around 50% beta-sheet structure in aqueous buffer with comparatively little (5–13%) alpha-helix. In the presence of helix promoters such as trifluoroethanol (TFE), the percentage alpha-helix rises to 33 to 45%, most of this increase being apparently due to random coil taking up this conformation. In an oil–water emulsion, peptide 1 seems to adopt alpha-

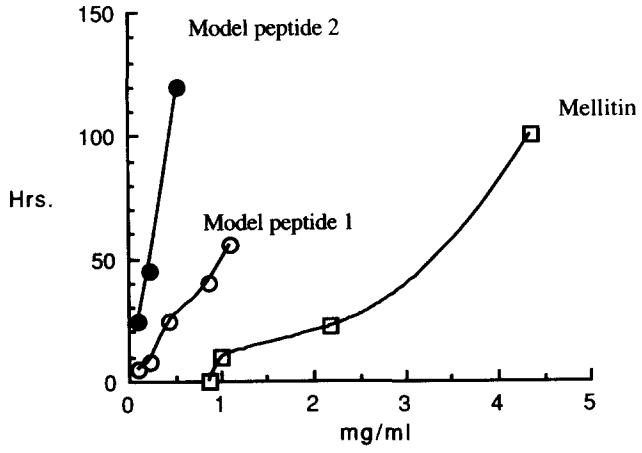

Fig. 1. Emulsification properties of model peptides 1 and 2 in an oil–water system. Time required for separation of the oil and water phases in the presence of various concentrations of mellitin (□), model peptide 1 (○) and model peptide 2 (●).

helix (27%) more readily than does peptide 2 (11%). These data indicate that the peptides adopt a range of ordered secondary structures rather than one single conformation. Their behaviour in oil–water emulsions cannot therefore be interpreted as due to any single structural feature such as beta sheet or alpha-helix.

From these studies it would seem that the emulsification properties of synthetic peptides arise from a combination of amphiphilicity and ordered secondary structure. We are presently extending this work with synthetic peptides possessing a broad range of ordered secondary structures in an attempt to correlate this with emulsification properties.

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