

INFLUENCE OF CASEIN PEPTIDE CONFORMATIONS ON TEXTURAL PROPERTIES OF CHEESE

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INTRODUCTION

An important relationship exists between casein proteolysis and the development of texture in cheese (Fox, 1989). Breakdown of caseins may begin as the curd is formed and continues through the ripening stage; it also occurs during refrigerated storage of non-ripened cheeses such as low-fat, high-moisture Mozzarella (Tunick et al., 1991; Malin et al., 1993). Renneting enzymes and plasmin provide casein fragments that eventually become substrates for internal proteases and peptidases that starter culture microorganisms use for metabolic benefit (Kok, 1990). Earlier investigations of proteolysis focused on ripened cheeses such as Cheddar or Gouda because the resulting peptides are necessary for texture and flavor attributes. Casein proteolysis has now proved to be a significant factor in development of texture and meltability in low-fat, high-moisture Mozzarella cheese (<10% fat) because of the correlation of these characteristics with breakdown of α_{s1} -casein (Tunick et al., 1993a, 1993b; Malin et al., 1994; see also Chapters 2 and 14).

Recognition of the influence of casein breakdown on texture has not yet brought a clear understanding of the mechanisms involved. An adequate explanation for the observed influence of casein breakdown on texture will depend on characterizing the cheese matrix and its components in molecular terms. Some models of the matrix consider it a composite material or filled gel and propose that the role of fat globules is to break up the solidity of the cheese network (Green et al., 1981; Walstra et al., 1987); successful fat substitutes perform the same function (Desai and Nolting, Chapter 17). As an extension of this view of the cheese matrix, the breakdown of the casein structure into smaller peptides may also be viewed as reducing the rigidity of the matrix network and providing greater flexibility. This hypothesis can be tested by probing the structure of caseins within the matrix. The recent work of Farrell et al. (1993) and Kumosinski et al. (1994a,b) in sequence-based

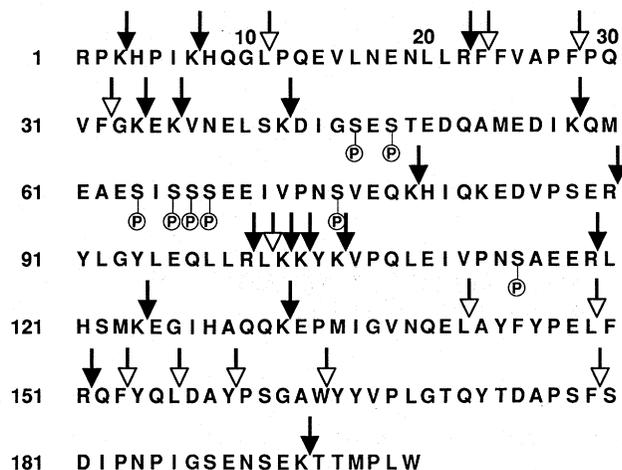


Figure 1. Sequence of α_{s1} -casein B showing cleavage sites of chymosin (Mulvihill and Fox, 1979; McSweeney et al., 1993a) as open arrows and cleavage sites of plasmin (McSweeney et al., 1993b) as solid arrows. The single letter code is used for amino acids.

predictions of the tertiary structures of caseins provides an opportunity to consider the conformations of peptides that result from the action of rennet or plasmin or both.

The work described here is a completely hypothetical prediction of the structures of peptides that might result when they are created by the action of chymosin and/or plasmin on α_{s1} -casein. It is based on cleavage sites reported by McSweeney et al. (1993a, 1993b) for the sequence of α_{s1} -casein (Eigel et al., 1984) as shown in Figure 1.

EXPERIMENTAL

The structures of α_{s1} -casein peptides were simulated with the molecular modeling program SYBYL, Version 6.0 (Tripos Associates,¹ St. Louis, MO) on a work station (SGI 4D/35, Silicon Graphics, Mountain View, CA). All peptides were subjected to energy minimization to find low-energy stable structures, refined further by one or more molecular dynamics simulations, and minimized again to smooth small perturbations created by the dynamics operation. This algorithm was repeated until each peptide energy was reduced to -7 to -11 kcal/mole/residue. The general procedure is outlined in Figure 2.

The premise of this approach is that a sequence of amino acids, regardless of length, can fold into a low-energy tertiary structure dictated by the spatial, electrostatic, hydrophilic, and hydrophobic requirements of its primary sequence (Anfinsen et al., 1973). The conformation of the sequence when it was part of α_{s1} -casein will not necessarily be retained, although existing structural features that satisfy the above requirements are likely to be modified. Each peptide investigated was excised as a segment from the computer display of the refined structure for the parent α_{s1} -casein predicted by Kumosinski et al. (1994b). All secondary and tertiary attributes

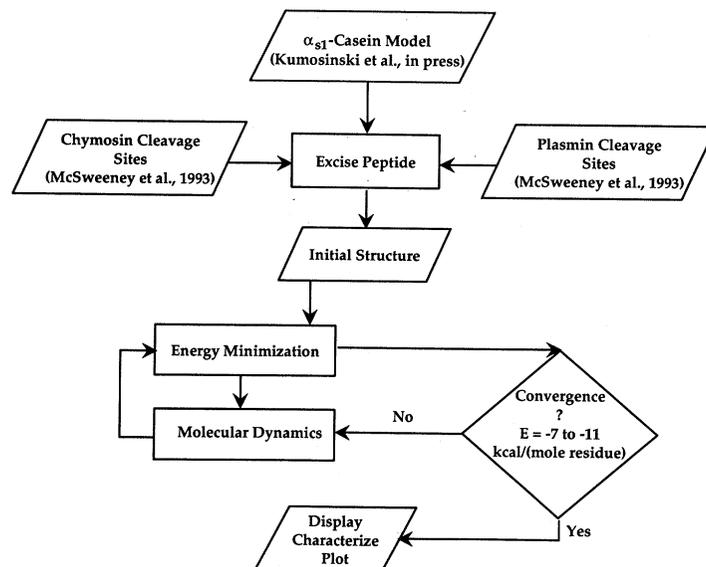


Figure 2. Flow chart of the procedure for excising a peptide from α_{s1} -casein and refining its structure to reach a low-energy stable conformation.

of that segment in the original α_{s1} -casein model were included. After the minimization procedures, analytical subroutines of the SYBYL modeling software were used to compare structural features of each peptide before and after minimization: number of hydrogen bonds, distance between α -carbons of the amino and carboxyl termini, and type and extent of secondary structure, if present.

RESULTS

Five of the seventeen peptides investigated are shown in Figures 3-7 as examples of the structural changes predicted by molecular modeling. For most of these only the peptide backbones are shown, without the residue sidechains that can obscure the general outline. Phosphoserine groups are shown as solid balls to call attention to the parts of α_{s1} -casein sequence that bind Ca^{2+} . Table 1 summarizes the changes in structural characteristics that are predicted to occur as the peptides fold into new structures.

Cleavage by Chymosin

The first cleavage of α_{s1} -casein by chymosin occurs between phe-23 and phe-24 (Mulvihill and Fox, 1979; McSweeney and Fox, 1993a); the peptide composed of residues 1-23 is shown in Figure 3a with its original conformation (Kumosinski et al., 1994a) and in Figure 3b after the refinement procedure described above. Although the representations of Figure 3 do not suggest a significant reduction in over-all size, Table 1 shows that the number of hydrogen bonds has almost doubled, indicating a more compact structure; this conformation is also more stable, as borne out by the energy which has undergone a four-fold reduction.

Figure 4 shows the structures of residues 33-101 before and after refinement as a separate peptide. This peptide also can result from chymosin cleavage; the sites

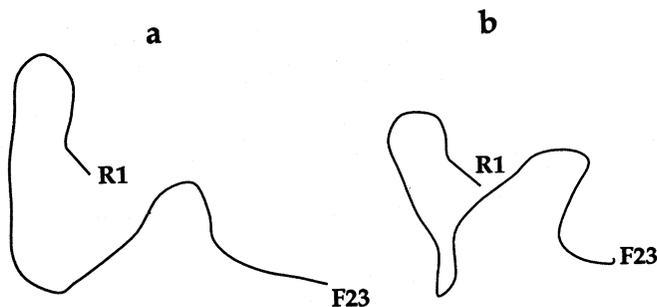


Figure 3. Main chain outline of α_{s1} -casein peptide composed of residues 1-23 created by chymosin cleavage between phe-23 and phe-24. a. Conformation as in the α_{s1} -casein model. b. Conformation after refinement.

are phe-32—gly-33 and leu-101—lys-102. The number of hydrogen bonds is increased by 50% after refinement, and the energy is reduced about eight-fold, indicating increased stability. However, defined secondary structure is decreased because of the loss of a small segment of β -sheet (Table 1).

Cleavage by Plasmin

Residues 106-151 of α_{s1} -casein (Figure 5) can result from the actions of plasmin at lys-105—val-106 and arg-151—gln-152 (McSweeney et al., 1993b). Table 1 shows that the number of hydrogen bonds has almost tripled and the energy has undergone a seven-fold reduction as a result of the refinement procedure. Existing α -helix is shifted by a few residues (from 125-128 to 132-134), and a small segment of β -sheet is converted to a ribbon conformation.

Cleavage by Chymosin and Plasmin

Formation of α_{s1} -casein peptides is not restricted to the action of only one protease. Plasmin is always found in milk, and chymosin (or other rennet) is added in cheesemaking. Therefore, there is a possibility of peptide formation by the action of both enzymes. Figure 6 shows residues 91-101 that might result from the action of plasmin at arg-90—tyr-91 and the action of chymosin at leu-101—lys-102 (McSweeney

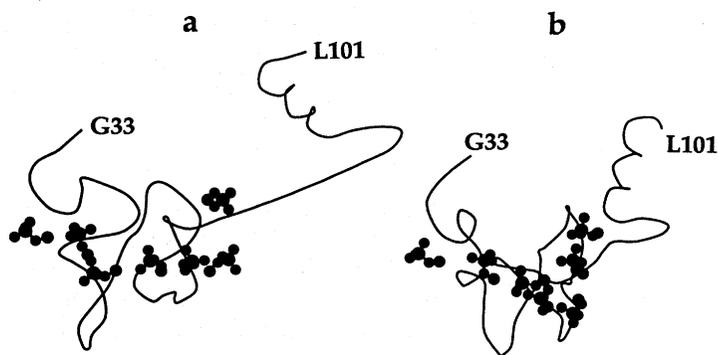


Figure 4. Main chain outline of α_{s1} -casein peptide composed of residues 33-101 created by chymosin cleavage between phe-32 and gly-33 and between leu-101 and lys-102. Phosphoserine groups are shown as solid balls to emphasize changes in spatial distribution that result from the refinement. a. Conformation as in the α_{s1} -casein model. b. Conformation after refinement.

Table 1. Effect of refinement on energies and structural features of α_{s1} -casein peptides. Initial = as excised from the refined model of α_{s1} -casein (Kumosinski et al., 1994b); final = after refinement (including minimizations and dynamics).

Peptide	No. Res.	Energy ^a		Hydrogen Bonds		C α -C α Distance, ^b Å		α -Helix		β -Sheet		Turns/Loops ^c	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1-23	23	-55.2	-221.7	15	28	18.2	15.5	0	0	0	0	4	3
33-101	69	92.9	-630.6	63	91	28.3	28.7	91-99	91-100	82-84	0	4	4
106-151	46	57.4	-408.8	23	60	34.4	32.7	125-128	132-134	136-144	137-139 ^d	3	5
91-101	11	-33.9	-104.5	8	13	14.7	14.0	91-99	91-101	0	0	1	0
143-151	9	28.3	-70.4	4	13	5.7	8.4	0	0	143-145	143-145 ^d	1	3

^aKcal/mole/residue. ^bDistance between α -carbons of amino and carboxyl ends of peptide; in peptides 33-101, 91-101, and 143-151 peptide ends had spread apart during refinement to satisfy energy requirements, but other portions of the peptides were more compact. ^cTurns or loops in the final conformation did not have similar conformation in initial structure. ^dResidues were in ribbon conformation.

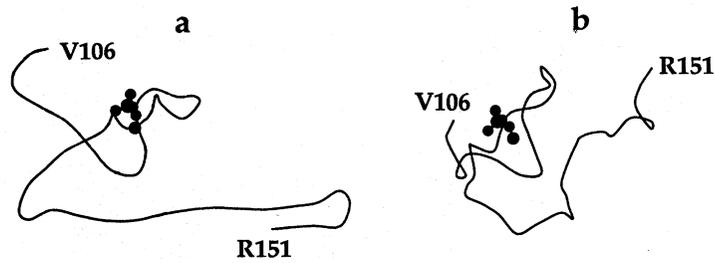


Figure 5. Main chain outline of α_{s1} -casein peptide composed of residues 106-151 created by plasmin cleavage between lys-105 and val-106 and between arg-151 and gln-152. Phosphoserine groups are shown as solid balls to emphasize changes in spatial distribution that result from the refinement. a. Conformation as in the α_{s1} -casein model. b. Conformation after refinement.

et al., 1993a, 1993b). Although this sequence already contained 82% α -helix within the whole α_{s1} -casein, Table 1 shows that the refined sequence was 100% α -helix. The number of hydrogen bonds was about 50% larger, and the energy had decreased almost four-fold.

Figure 7 shows the peptide composed of residues 143-151 that could be formed by the action of chymosin at leu-142—ala-143 and of plasmin at arg-151—gln-152 (McSweeney, et al., 1993a, 1993b). The sidechains of arg-151 and glu-148 are shown to highlight the dramatic change when ionic bonding (salt bridges) occurs between residues. Some of the hydrogen bonds are also shown to emphasize the structural stabilization resulting from the three-fold increase in number of hydrogen bonds predicted by the refinement. The energy was reduced about four-fold.

DISCUSSION

In the peptides of Figures 3-7, as in twelve other peptides not shown, there was a strong tendency to adopt more compact, stable structures as chain lengths decreased. These observations may explain the decrease in hardness and increase in meltability found in low-fat, high-moisture Mozzarella (<10% fat) that accompany substantial proteolysis of α_{s1} -casein during 6 wk of refrigerated storage (Tunick et al., 1991; Malin et al., 1993; Chapters 2 and 14).

Application of materials science concepts to the structure of cheese has led to the filled gel or composite model of the cheese matrix (Green et al., 1981; Walstra et

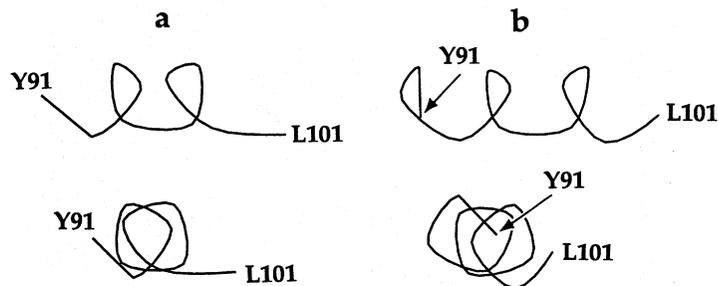


Figure 6. Main chain outline of α_{s1} -casein peptide composed of residues 91-101 created by plasmin cleavage between arg-90 and tyr-91 and by chymosin cleavage between leu-101 and lys-102. a. Side view (above) and end view (below) of conformation as in the α_{s1} -casein model. b. Side view (above) and end view (below) of conformation after refinement.

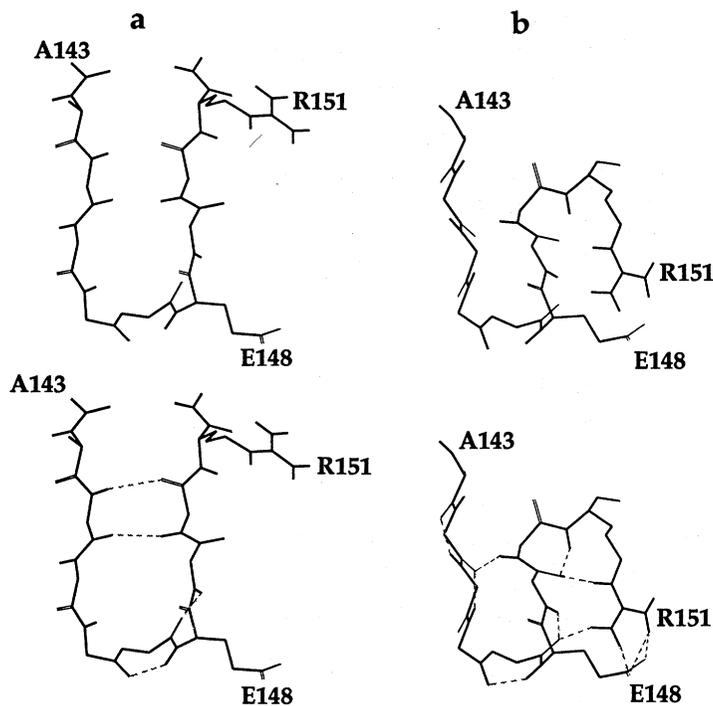


Figure 7. Backbone of α_{s1} -casein peptide composed of residues 143-151 created by chymosin cleavage between leu-142 and ala-143 and by plasmin cleavage between arg-151 and gln-152. a. Conformation as in the α_{s1} -casein model showing the side chains of arg-143 and glu-148. b. Conformation after refinement showing the ionic interaction between arg-143 and glu-148. Major hydrogen bonding sites are shown in the lower half of the figure to indicate how refinement stabilizes the more compact structure.

al., 1987; Desai and Nolting, Chapter 17). In this view, the fat globules distributed throughout the cheese act as fillers in the protein network and have a direct influence on textural and mechanical properties. In the low-fat, high-moisture Mozzarella, with a fat content only 41% of that in full-fat Mozzarella, the number of fat droplets is insufficient to provide the texture and mechanical properties of a full-fat cheese. However, the small, compact peptides formed during substantial proteolysis may act as fillers in place of fat globules.

In the low-fat, high-moisture Mozzarella cheese (Tunick et al., 1991; Malin et al., 1993, 1994; Chapters 2 and 14), the casein matrix undergoes substantial proteolysis during a 6-wk storage period— α_{s1} -casein content is reduced by 40 to 50% after 6 wk of storage. Smaller, more stable, more compact structures could more easily move aside upon the application of force, as in tests of hardness. Fat droplets in the cheese would be less protected during heating from the insulating effect of a more dense cheese matrix, and the more open structure of the cheese matrix would promote increased meltability by providing more freedom for the flow of fat within the matrix.

The development of compact structures by α_{s1} -casein peptides may also explain the image-analyzed electron micrographs of Cooke et al. (Chapter 19) that indicate a significant change in the distribution of electron dense areas during the 6 wk storage of low-fat, high-moisture Mozzarella cheese. Kumosinski et al. (1994c) have proposed a casein submicelle model based on the refined tertiary structures of caseins predicted by molecular modeling (Farrell et al., 1993; Kumosinski et al., 1994a,b). When viewed from several different angles, the outlines of this casein micelle model are strikingly

similar to electron micrographs of casein submicelles (Farrell et al., 1994). Loss of portions of the casein components of the micelle, through proteolysis by chymosin or plasmin, is predicted by the model to result in changes in electron density similar to those observed by Cooke et al. (Chapter 19) in image-analyzed micrographs of 6-wk Mozzarella cheese.

The results of molecular modeling of α_{s1} -casein peptides indicate considerable potential for further investigations of the properties of the cheese matrix and may provide data supporting the role of proteolysis in developing texture in low-fat cheese.

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