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Structural Relatedness of κ-Casein and Fibrinogen γ-Chain

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Summary. κ- Caseins, involved in the milk clotting process, and human fibrinogen γ-chain, involved in the blood clotting process, show structural similarities. Several long κ-casein sections, together corresponding to 80% of the whole protein molecule, have their counterparts in the γ-chain of fibrinogen, in that 31-42% of the amino acid residues occupy identical positions. The section of κ-casein which contains the chymosin-sensitive bond has a counterpart not only in the γ but also in the Bβ-chain of fibrinogen. Furthermore, the secondary structures of the κ-caseins and of the γ-chain predicted according to the method of Chou and Fasman present several common features.

Recently Jollès et al. (1974) and Jollès (1975) suggested a structural relationship between the milk and blood clotting processes. Among the various common features, it was demonstrated that certain amino acid sequences of cow and sheep κ-caseins, which are substrates of chymosin (EC 3.4.23.4) and of human fibrinogen, which is a substrate of thrombin (EC 3.4.21.5), present homology. The complete primary structures of cow (Brignon et al., 1972; Jollès et al., 1972; Mercier et al., 1972; Jollès et al., 1973) and sheep (Jollès et al., 1974) κ-caseins have been known for several years, whereas only the C-terminal part of human κ-casein was so far established (Chobert et. al., 1976; Fiat et al., 1977). The first complete sequence of a fibrinogen chain, the γ-chain of human fibrinogen, became available (Henschen et al., 1976; Lottspeich and Henschen, 1977a, b) only quite recently. Thus it was now possible to extend the structural comparisons and to demonstrate a closer homology between large parts of the amino acid sequences of the κ-caseins and the γ-chain. The secondary structures of the κ-caseins (Loucheux-Lefebvre et al., 1978) and of the γ-chain of human fibrinogen were predicted to allow further comparisons of biologically important sites in the two kinds of molecules. Hereby some additional common features became apparent.

*41th communication on caseins.

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Prediction of Secondary Structure

The predictive rules formulated by Chou and Fasman (1974) were applied to predict the secondary structures. The assignment of residues in terms of helical or β-sheet potentials were obtained using the parameters given by Fasman et at., (1976). The β-turns were predicted using the bend positional potentials and β-turn conformational parameters evaluated by Chou and Fasman (1977) in a recently published survey.

Results and Discussion

Homology Between the Primary Structures of Human, Cow and Sheep κ-caseins and Human Fibrinogen (γ-chain)

Table 1 reports six sections in these proteins presenting homology. The homologous peptides are rather large (16-37 amino acids) and have 31 to 42% of their amino acids in identical positions. Some points should be emphasized. (a) The cysteine residues (No 10, 11 and 88) of the κ-caseins are in register with cysteine residues of the γ-chain (Table 1, i and iv); (b) The casein sequences containing the prosthetic sugar group, i.e. the threonine residue No 135 for sheep- or No 133 for cow κ-caseins is homologous to a erpart in a section of the γ-chain of fibrinogen which is, however, devoid of carbohydrate (Table 1, v). In a similar way, the carbohydrate carrying asparagine residue No 52 of the γ-chain is located in a sequence showing homology with cow κ-casein which here is devoid of carbohydrate (Table 1, iii). (c) A long sequence surrounding the important chymosin-sensitive bond of cow and sheep κ-caseins is homologous to a sequence in the γ-chain of fibrinogen (Tavle 1, iv). A short, recently sequenced fragment of human κ-casein (Chobert et al., 1976; Fiat et al., 1977) showed the same degree of homology with the γ-chain as the cow and sheep κ-caseins, though the identical residues were partly found in different positions (Table 1, iv). In κ-caseins, the sensitive bond is situated between residues No 105 and 106, which divide the molecule into two moieties: a hydrophobic, rather ordered moiety and a hydrophilic, less ordered one. The corresponding bond in the γ-chain of fibrinogen is situated between residues No 168 and 169 which also can be regarded as defining two different parts of the molecule as shown by the studies devoted to the secondary structure (see below). Chymosin splits only a unique peptide bond in κ-caseins (No 105 → 106) during the primary phase of the milk clotting process. However under similar experimental conditions it attacks the γ-chain of fibrinogen much more easily, rapidly splitting several peptide bonds. (d) The sequence 143-164 in cow κ-casein is homologous to two different γ-chain sequences (36-57 and 128-145; Table 1, iii). The same is true for a sheep κ-casein sequence from the same region. Furthermore, the sequence 42-57 of the γ-chain is homologous to two different parts of cow (69-83 and 149-164) as well as sheep (69-83 and 151-166) κ-casein. (e) All homologies between κ-caseins and the γ-chain of fibrinogen are limited to the N-terminal half of the γ-chain.

Table 2 summarizes the homologies occurring betweeen cow κ-casein and the γ-chain of human fibrinogen: 80% of the κ-casein molecule contained in 3 large fragments (one of them being 79 amino acid residues long) is homologous to 6 large γ-chain fragments. In the case of sheep κ-casein the situation is almost the same: 77% of the protein is homologous to peptides encoutered in the γ-chain.

Table 1. Sequence homology between the γ- (Henschen et al., 1976; Lottspeich and Henschen, 1977a,b) and Bβ- (Henschen and Lottspeich, 1977a) chains of human fibrinogen and cow- (Brignon et al., 1972; Jollès et al., 1972; Mercier et al., 1972; Jollès et al., 1973), sheep- (Jollès et al., 1974) and human (Chobert et al., 1976; Fiat et al., 1977) κ-caseins. The one-latter amino acid abbreviation system was employed. n.d. not determined. The homologous residues between the γ-chain of fibrinogen and the κ-casein are boxed: □ identical amino acids; ⋯ conservative changes. * carbohydrate carrying residue; –, gap

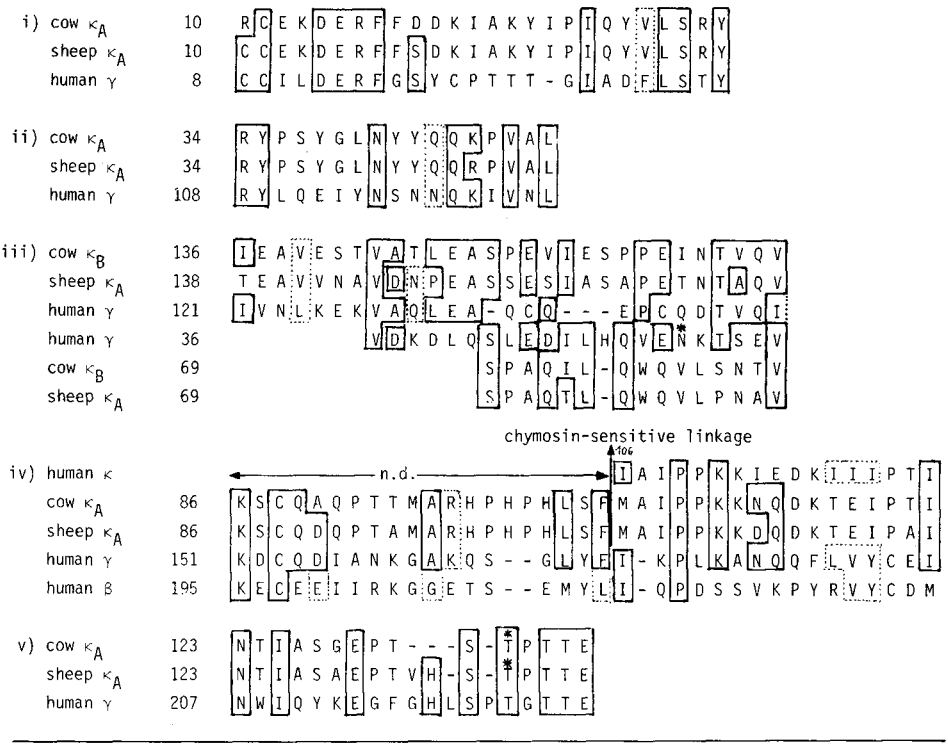
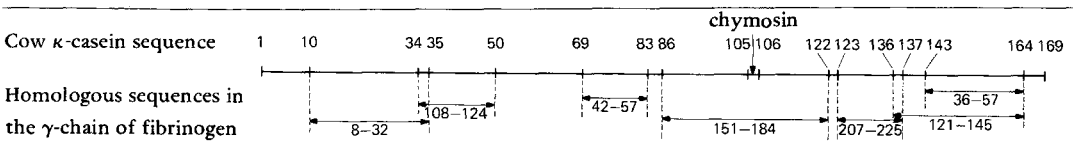


Table 2. Schematic representation of cow κ-casein and of the regions presenting homology with the γ-chain of human fibrinogen



Number of positions compared	26	17	16	37	19	29 or 22
% identical amino acids	35	41	31	30	42	34 or 31

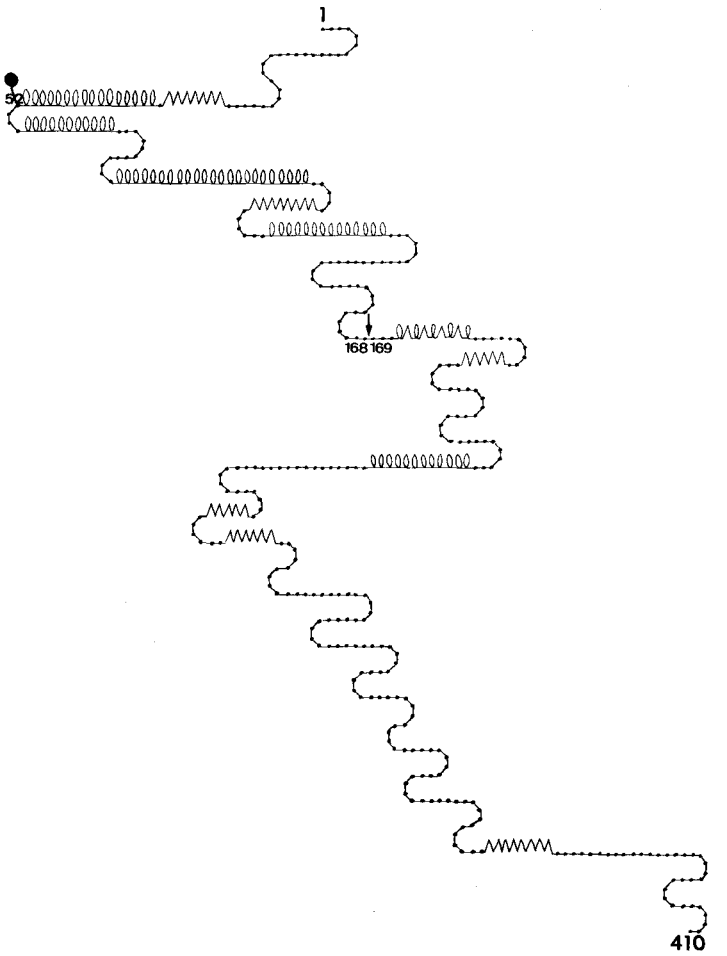


Fig. 1. Schematic diagram of the predicted secondary structure of the γ-chain of human fibrinogen. Residues are represented in their respective conformational state: helical (◊), β-sheet (Λ), section which has the potentiality to adopt either an α-helical or a β-sheet structure (◊Λ), coil (-), β-turn (□). ⥉, carbohydrate carrying residue. ↑ bond corresponding to the chymosin-sensitive bond in κ-casein (see text)

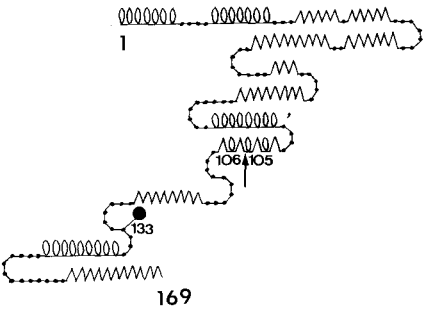


Fig. 2. Schematic diagram of the predicted secondary structure of cow κ-casein. The symols have same meaning as in Fig. 1, except for ↑, which represents the chymosin-sensitive bond

Predicted Secondary Structures of the γ-chain of Human Fibrinogen and of Cow κ-Casein

The complete predicted secondary structures of both proteins with the α-helix and β-sheet regions as well as the β-turns are shown in schematic diagrams (Fig. 1 and Fig. 2).

Common Features Between the Predicted Secondary Structures of Cow and Sheep κ-Caseins and the γ-Chain of Human Fibrinogen

Figure 1 clearly shows that the N-terminal part of the γ-chain of human fibrinogen has a rather ordered structure with several long α-helical regions and some β-sheets. A similar situation is found in the N-terminal moieties of cow (Fig. 2) and sheep (not shown) κ-caseins (Loucheux-Lefebvre et al., 1978).

Not only the primary structure but also the secondary structure of the γ-chain in the region residues No 168 and 169 shows homology with the κ-caseins in the region of the chymosin-sensitive bond (residues 105 → 106). In both proteins these residues are situated right after a β-turn and either just before or within a section which has the potentiality to adopt either an α-helical or a β-sheet structure. Such a conformation has previously also been found in the proximity of the thrombin-sensitive bonds in the Aα- and Bβ-chains of fibrinogen.

It is worthwhile mentioning that the carbohydrate-peptide linkage of the γ-chain of fibrinogen (residue No 52) was found to be situated in a β-turn as the majority of the previously described analogous linkages in other proteins (Aubert et al., 1976; Loucheux-Lefebvre et al., 1978).

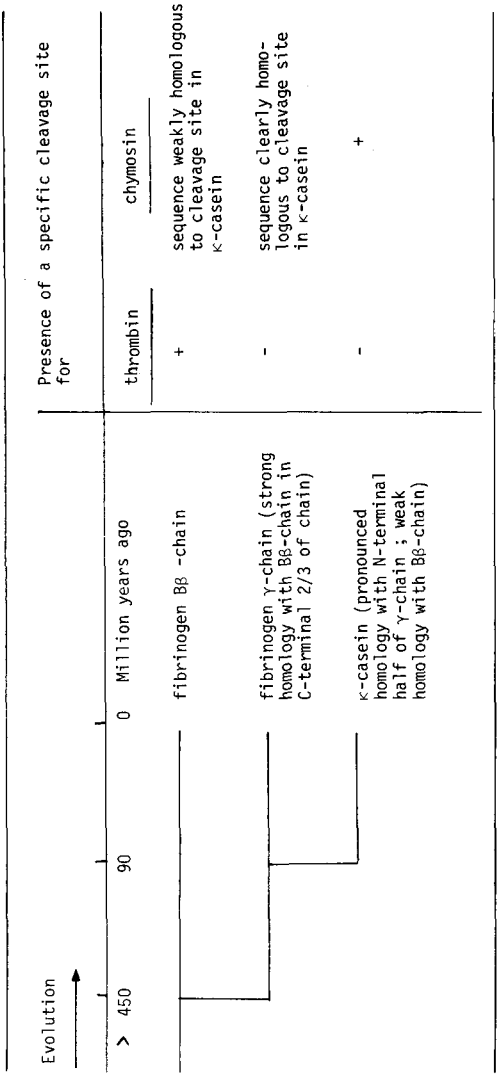
The C-terminal part of the γ-chain of fibrinogen (beyond residue 225) contains no more sequences homologous to those of κ-caseins. This part of the molecule presents a secondary structure with a high percentage of β-turns and unordered structure (Fig. 1), which may be explained by the relatively high glycine content (Rajan and Srinivasan, 1976). In fact, 73% of the glycine residues belong to a β-turn and are mainly situated at the third and fourth position of the β-turns, as expected from the data of Chou and Fasman (1977).

Conclusion

A pronounced homology was observed for the primary structures of cow and sheep κ-caseins and the γ-chain of human fibrinogen (Tables 1 and 2). At present it is not possible to understand why the homologous sections are found in a different order and partly duplicated in κ-casein as compared to fibrinogen γ-chain (Table 2). The secondary structures of these two kinds of proteins presented some additional common features. The γ-chain is not attacked by thrombin but contains a sequence corresponding to the chymosin-sensitive sequence of the human, cow and sheep κ-caseins.

As the Bβ- and γ-chains of human fibrinogen are clearly homologous (Henschen et al., 1976; Henschen and Lottspeich, 1977a,b; Lottspeich and Henschen, 1977a,b), it was no surprise to find some structural homologies also between the κ-caseins and the Bβ-chain. However, these homologies are much less pronounced than those with the γ-chain. The N-terminal sequences (1–15) of both κ-caseins have earlier been shown to be homologous to the sequence 83–99 of the Bβ-chain (Jollès et al., 1974; Jollès, 1975). Moreover the Bβ-chain contains a sequence (195–228) related to the long κ-casein fragment (86–122) containing the chymosin-sensitive linkage (Table 1, iv), though the resemblance is here much less obvious than with the γ-chain.

Table 3. Tentative evolutionary relationship between the Bβ- and γ-chains of fibrinogen and the κ-caseins.[a]



Presence of a specific cleavage site for		
	thrombin	chymosin
fibrinogen Bβ -chain	+	sequence weakly homologous to cleavage site in κ-casein
fibrinogen γ-chain (strong homology with Bβ-chain in C-terminal 2/3 of chain)	-	sequence clearly homologous to cleavage site in κ-casein
κ-casein (pronounced homology with N-terminal half of γ-chain ; weak homology with Bβ-chain)	-	+

[a] The Aα-chain is not included as its complete structure is not yet known

The hypothetical evolutionary relationship between fibrinogen and κ-casein is depicted in Table 3. The following aspects have been taken into consideration: fibrinogens, composed of three different peptide chains, seem to occur in all vertebrates. The strong homology between the Bβ- and γ-chain indicates that they have a common ancestor, which then should have existed more than 450 million years ago. Caseins must be relatively young proteins, as they are milk proteins and mammals only have existed for about 90 million years. As the κ-caseins have appeared much later during evolution than the fibrinogens it seems reasonable that they could have evolved from only one of the fibrinogen chains, i.e. the γ-chain. The fact that the two fibrinogen chains still are much similar to each other than to the κ-casein might be regarded as an expression of evolutionary constraint and divergence, respectively, guided by adaptation to the

differences in function of the two types of protein. However, it should be strongly emphasized that further studies are necessary in order to give more insight into the evolutionary and biological significance of the observed structural homologies.

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