COMPARATIVE STUDY OF COW AND SHEEP κ-CASEINOGLYCOPEPTIDES: DETERMINATION OF THE N-TERMINAL SEQUENCES WITH A SEQUENCER AND LOCATION OF THE SUGARS*

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Received 5 January 1973

1. Introduction

κ-Casein plays a major role in the stabilization of the casein micelle in its natural environment [1] and in the clotting phenomenon induced by the action of rennin (EC 3.4.4.3); it is also the only casein fraction that contains sugars [2, 3]. To the heterogeneity of κ-casein from pooled milk are contributing the genetic variants, but also the non-identical composition of the carbohydrate groups present. This observation is in accordance with Gottschalk's [4] concept on the heterogeneity of the carbohydrate group in glycoproteins. During the rennin clotting of milk a Phe–Met bond is split [5]. A large peptide, called κ-macropeptide when κ-casein is sugar-free or κ-caseinoglycopeptide when κ-casein is sugar-rich, is liberated and para-κ-casein, devoid of sugars, precipitates. Bovine κ-caseinoglycopeptide has already been submitted to extensive structural studies [6–9] but only few data are available for sheep κ-caseinoglycopeptide [5, 10, 11]. The present note reports the determination of the N-terminal sequence of the latter (40 amino acid residues) with a sequencer and some comparisons with cow κ-caseinoglycopeptide; furthermore the location of the short glycopeptide obtained independently from cow κ-casein by enzymic procedures is discussed.

* 30th communication on caseins.

2. Material and methods

Cow κ_A-casein was prepared according to McKenzie and Wake [12] from the milk of homozygous cows and sheep κ-casein according to Alais and Jollès [13]. The κ-caseinoglycopeptides were obtained after rennin digestion of the corresponding κ-caseins as previously described [14].

Automated Edman degradation [15] was carried out in a Beckman sequencer, Model 890 B, by the quadrol method. The thiazolinones were converted into PTH-amino acids and these latter were characterized by thin-layer chromatography, by gas-liquid chromatography (Beckman GC 45 chromatograph) or with an amino acid autoanalyzer after regeneration of the free amino acid.

Short glycopeptides were isolated from cow and sheep κ_A caseins by enzymic digestions (neuraminidase, EC 3.2.1.18; chymotrypsin, EC 3.4.4.5; pronase) and further purified by filtration on Sephadex G-25 and paper electrophoresis according to Fiat et al. [16]. Their structures were established mainly by the manual Edman technique.

3. Results

3.1. *N-terminal sequences of cow and sheep κ_A-caseinoglycopeptides (38 and 40 residues, respectively) determined with a sequencer*

Table 1 indicates in detail the establishment of the

Table 1

N-terminal sequences of cow and sheep κ_A-caseinoglycopeptides determined with a sequencer.

Cow *	1	Met	Ala	Ile	Pro	Pro	Lys	Lys	Asn	Gln	Asp	Lys	Thr	Glu		
Sheep**	1	Met	Ala	Ile	Pro	Pro	Lys	Lys	Asp	Gln	Asp	Lys	Thr	Glu		
(a)		+	+	+	+	+	+	+	+	+	+	+	+	+		
(b)			24	26	20		16	22	5	10		10		7		
(c)		40	36	12	15	7										

Cow *	14	Ile	Pro	Thr	Ile	Asn	Thr	Ile	Ala	Ser	Gly	Glu	Pro	Thr	Θ	Θ
Sheep**		Ile	Pro	Ala	Ile	Asn	Thr	Ile	Ala	Ser	Ala	Glu	Pro	Thr	Val	His
(a)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	***
(b)		7.7	5	12.5	5	3		8	9		7	6	2.7		6	
(c)		10	4	10							5				5	

Cow *	27	Ser	Thr	Pro	Thr	Ile	Glu	Ala	Val	Glu	Ser	Thr	Val
Sheep**	29	Ser	Thr	Pro	Thr	Pro	Glu	Ala	Val	Val	Asn	Ala	Val
(a)		+	+	+	+	+	+	+	+	+	+	+	+
(b)				1.5			3.5	3.2	3	3.8	1.3	1	0.7

Underlined amino acid: change between sheep and cow caseinoglycopeptides. Surrounded sequence: sugar containing peptide obtained by enzymic digestions.

* Experimental details, see Jollès et al. [8]; PTH-Ser and PTH-Thr characterized by thin-layer chromatography.

** Experimental details for the sheep peptide: (a) PTH-amino acid determined by thin-layer chromatography; (b) amino acid determined with an autoanalyzer after regeneration, % recovery; (c) PTH-amino acid determined by gas-liquid chromatography, yield %.

*** Characterized with the Pauli reagent after electrophoresis.

To optimize homologous relationships, two deletions were suggested to occur in the cow peptide. Θ: deletion.

N-terminal sequences. To optimize homologous relationships, two insertions were suggested to occur in the sequence of the sheep peptide including particularly the unique histidine residue, when compared to the cow peptide [8]. The sequence concerning this latter is in accordance with the results published by Mercier et al. [9]. The N-terminal sequence of the sheep peptide contained the previously described chymotryptic peptide: Thr–Ile–Ala–Ser–Ala–Glu–Pro–Thr–Val–His [17].

3.2. *Location and attachment site of the short glycopeptide obtained from cow κ_A-casein*

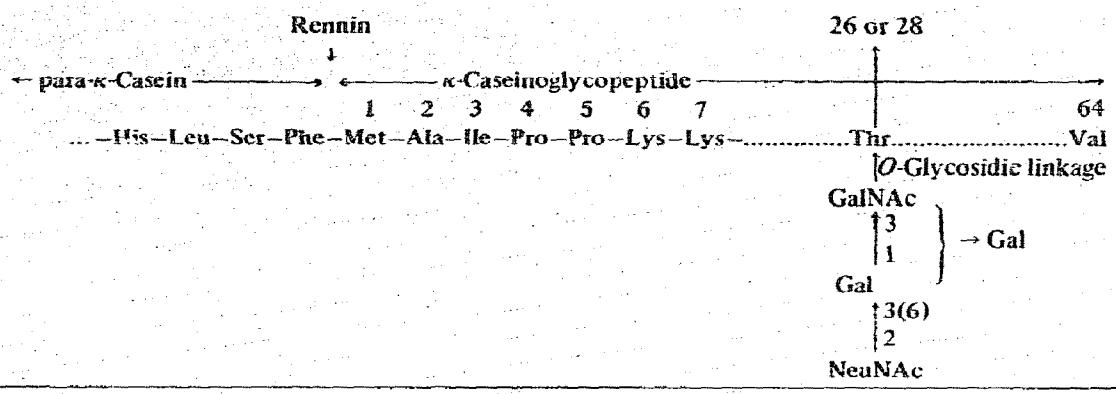
The glycopeptide fraction (mobility m = −0.45 at pH 6.5) prepared according to Fiat et al. [16] from cow κ_A-casein contained two peptides; they were submitted to the Edman technique and the PTH-amino acids were characterized by gas-liquid chromatography. By this sensitive procedure, it was established that the main glycopeptide containing the unique Gly residue of the molecule, Gly–Glu–Pro–Thr–Ser–Thr–Pro–Thr (residue 23–30), was accompanied by another, less abundant peptide, Ser–Gly–Glu–Pro–Thr–Ser–Thr–Pro (residues 22–29) [8].

The κ-caseinoglycopeptides contain an *O*-glycosidic linkage between GalNAc and a residue of threonine [18]. The attachment site of the polysaccharide could not be the C-terminal Thr residue (no. 30), as a β-elimination was observed [19]. Thus threonine residues no. 26 and no. 28 might be linked to the sugar part. However, if Thr–X–X–Pro (X = amino acid) might be suggested as "code sequence" for an *O*-glycosidic linkage between Thr and *N*-acetylgalactosamine [8], then threonine no. 26 seems to be the most probable residue involved in the sugar linkage.

3.3. *The glycopeptide fraction of sheep κ_A-casein*

The glycopeptide fraction of sheep κ_A-casein (mobility m = −0.37 at pH 6.5) contained several substances; only two of them have been studied at the time being. The short peptide Ala–Glu–Pro–Thr (residues 23–26) was devoid of sugars as previously

Table 2
Schematic representation of the attachment site of the sugars in cow κ-casein.



reported [17]; the second peptide seemed to contain residues 29–35 as well as the sugars. Definitive conclusions cannot be drawn but it might be suggested that the sugar part in sheep κ-casein should be situated in the same area as in cow κ-casein. Here again, if Thr–X–X–Pro might be suggested as "code sequence", threonine residue no. 30 can be considered as a candidate for the attachment site of the polysaccharide.

4. Conclusion

4.1. *Schematic representation of the sugar part in cow κ_A-casein*

Previous studies have established the structure of the polysaccharide in cow κ-casein [16]:

$$\text{Neu NAc} \xrightarrow{2\quad 3(6)} \text{Gal} \xrightarrow{1\quad 3} \underbrace{\text{GalNAc}}_{\text{Gal}} \xrightarrow{O\text{-glycosidic linkage}} \text{Thr (A)}$$

An excess of galactose was always observed [16, 20]. Extensive digestions with β-galactosidase (EC 3.2.1.23) from *E. coli* (a gift from Prof. K. Wallenfels, Freiburg-im-Br.) gave rise to free Gal which might be linked either to Gal or to GalNAc of formula (A) [20]. Similar results were obtained with sheep κ-casein (A.-M. Fiat and P. Jollès, unpublished results). Table 2 gives a schematic representation of the structure of cow κ_A-casein with the most probable attachment site of the polysaccharide.

Acknowledgements

The excellent technical assistance of Miss F. Schoentgen, Mrs. M. Berger and Mr. Ly Quan Le is gratefully acknowledged. This research was supported in part by the C.N.R.S. (ER 102), D.G.R.S.T. (grant 72.7.0470) and I.N.S.E.R.M. (groupe U 116 and contrat libre 1972).

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