

Short Communication

Human αS_1 -Casein Like Protein: Purification and N-Terminal Sequence Determination

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Casein components of human milk are generally reported to belong to the β - and κ -groups. In this research human casein fraction was obtained from pooled mature milk, either by ultracentrifugation or by acid precipitation. In both cases a minor component with a slightly higher mobility in SDS-PAGE than β -casein was identified. The protein was purified to homogeneity, the N-terminal sequence of the first 14 amino acid residues of this new human casein subunit shows a high degree of homology with the αS_1 -casein sequences from other species.

Key words: Allergy / Casein / Homology / Human milk / Sequence.

Casein subunits are a heterogeneous group of milk proteins which associate into micelles with organic and inorganic ions. Caseins account for 80% of total protein content in cow milk, while in mature human milk they represent only the 30–35% of the total proteins (Anderson *et al.*, 1982; Lonnerdal, 1985).

Cow milk caseins are divided into three major classes: α -, β - and κ -casein, with different functional properties; up to now it has generally been accepted that the α -casein class is lacking in human milk (Azuma *et al.*, 1985; Brignon *et al.*, 1985), β -casein being the major component.

The main biological role of caseins has been thought to be simply that of a source of amino acids and a vehicle for phosphorous and calcium ion adsorption; more recently, very interesting functions for small peptides derived from proteolytic degradation of β -casein have been discovered (Koldovsky, 1989; Ramabadran and Bansinath, 1988). Similar studies on the physiological role of α -casein are very few, probably because this class of proteins is generally thought to be absent in human milk.

In this report we describe the identification, isolation and purification of a casein subunit from a pool of mature

human milk, whose N-terminal amino acid sequence is highly homologous with the αS_1 -casein sequences found in the milk of other species.

Casein pellets were obtained from a pool of human milk samples either by ultracentrifugation at $189\,000 \times g$ (2 h, 4 °C), or by precipitation with acetic acid at pH 4.3. The resulting pellets were washed in deionized water and lyophilized.

Analytical SDS-PAGE was performed in a LKB 2050 Midget Electrophoresis Unit according to Laemmli (Laemmli, 1970). The resolving gel was 12%T, 3%C and the stacking gel 4%T, 3%C; both gels were 0.75 mm thick.

Western blotting after SDS-PAGE was performed in a Midget MultiBlot Electrophoresis Transfer Unit: the minigels were blotted onto ProBlott membrane (Applied Biosystems) following Towbin (Towbin and Gordon, 1984), but methanol and SDS were omitted in the Transfer buffer. The membrane was stained in 0.1% SERVA blueG dissolved in the destaining solution (50:10:40 methanol : acetic acid : water). After a final wash in deionized water, the membrane was dried in air at room temperature.

Preparative SDS-PAGE was carried out in a SE 600 vertical slab gel Electrophoresis Unit (Hoefer) on 1.5 mm thick gels. Electrophoresis was performed according to Laemmli (Laemmli, 1970) but the pH of the gel buffer in the stacking matrix was 8.8; resolving gel was 12%T, 3%C and stacking gel 4%T and 3%C. After electrophoresis, small strips of the gel were cut from both edges of the slab, stained in Coomassie blue, destained and allowed to return to original size by immersion in the electrophoresis buffer. Corresponding bands were cut out of the non-stained gel and submitted to electroelution.

Electroelution and concentration of proteins after preparative SDS-PAGE was performed in a ISCO–1750 Electrophoretic Sample Concentrator. Each portion of the preparative gel was cut into small pieces and placed in the cup of the ISCO concentrator. Electroelution was carried out at 1 watt for 90' in the Laemmli SDS-PAGE run buffer, both in the cups and the electrode tanks. Amino acid sequences were determined on an Applied Biosystems Model 470A gas-phase sequencer equipped with an on-line Model 120A phenylthiohydantoin (PTH) derivatives analyzer (Hewick *et al.*, 1981).

Lyophilized casein pellets were solubilized with Laemmli sample buffer and analyzed by SDS-PAGE (Figure 1) and subsequent Western blotting. This afforded clear identification of each protein component, by their N-terminal sequences. β -casein, a phosphorylated protein of 212

amino acid residues (Greenberg *et al.*, 1984) was the main protein component of the casein pellet detectable by Coomassie staining (Figure 1). Along with two proteolytic fragments of β -casein, a component with an apparent M_r of 27 000 and R-P-K-L as N-terminal amino acids, not yet described in human milk, can clearly be seen in the electrophoretic pattern in Figure 1. By densitometric evaluation of the electrophoretic pattern, this new protein component represents about the 0.06% of the total protein content of human milk (19% of the β -casein content). In order to characterize this new protein of human casein pellet, a preparative SDS-PAGE, followed by electroelution of the separated fractions, was set up. On about 57 pmol of the isolated fraction corresponding to the new protein component, the first 14 N-terminal amino acid residues were identified by automated Edman degradation analyses (Table 1). Comparative studies with already

Table 1 N-Terminal Amino Acid Sequence Comparison of Human α s1-Casein Like Protein with already Known α s1-Casein Sequences from other Species.

	1	5	10	14
Human	R P K L	P L R Y	P E R L	Q N
Cow	R P K H	P I K H	Q G L P	Q E
Sheep	R P K H	P I K H	Q G L S	P E
Goat	R P K H	P I N H	Q G L S	P E
Rat	L P R A	H R R N	A V S S	Q T
Mouse	M P R L	H S R N	A V S S	Q T
Rabbit	R H K F	H L G H	L K L T	Q E

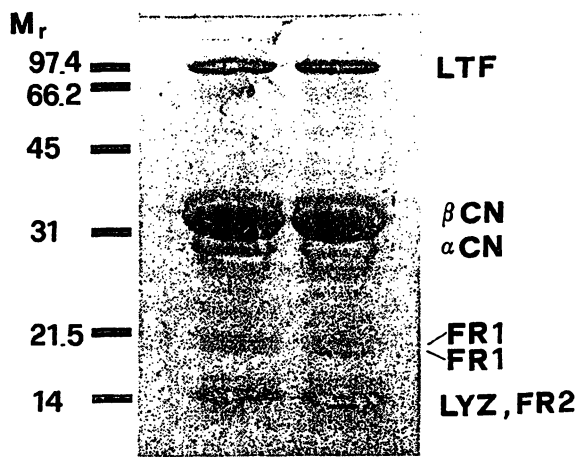


Fig. 1 Electropherogram of Human Casein Pellet Components. After SDS-PAGE and Western blotting on ProBlott membrane, samples were stained with SERVA blueG. M_r molecular mass markers in kDa; LTF: lactoferrin; β -CN: β -casein; α -CN: α s1-casein like component; FR1: β -casein fragment from aminoacid 1 onward; LYZ: lysozyme; FR2: β -casein fragment from amino acid 97 onward.

known sequences, showed a high level of homology with α s1-caseins from cow (Mercier *et al.*, 1971), sheep (Mercier *et al.*, 1985), goat (Brignon *et al.*, 1989), rat (Yu-Lee *et al.*, 1986), mouse (Grusby *et al.*, 1990) and rabbit (Devinoy *et al.*, 1988) milk. Comparison of human α s1-casein with N-terminal sequences of other species revealed several identical amino acids (Table 1). The first seven residues can be found in part either in the group of ruminants (Arg₁, Pro₂, Lys₃, Pro₅), or in rabbit (Arg₁, Lys₃, Leu₆), or in the group of mouse and rat (Pro₂, Leu₄, Arg₇). Lysine in position 3 seems to be a conserved residue, being replaced in rat and mouse only by Arginine, which belongs to the same exchange group of positively charged amino acids. The same character of conserved amino acid is observed for Glutamine in position 13. Gln 13 is present in all the species, except sheep and goat.

The isolation of higher quantities of this new α s1-casein present in human milk is in progress in order to determine its complete aminoacid sequence.

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