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Characterization of casein and alpha lactalbumin of African elephant (*Loxodonta africana*) milk

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ABSTRACT

The current research reports partial characterization of the caseins and α-lactalbumin (α-LA) of the African elephant with proposed unique structure-function properties. Extensive research has been carried out to understand the structure of the casein micelles. Crystallographic structure elucidation of caseins and casein micelles is not possible. Consequently, several models have been developed in an effort to describe the casein micelle, specifically of cow milk. Here we report the characterization of African elephant milk caseins. The κ-caseins and β-caseins were investigated, and their relative ratio was found to be approximately 1:8.5, whereas α-caseins were not detected. The gene sequence of β-casein in the NCBI database was revisited, and a different sequence in the N-terminal region is proposed. Amino acid sequence alignment and hydropathy plots showed that the κ-casein of African elephant milk is similar to that of other mammals, whereas the β-casein is similar to the human protein, and displayed a section of unique AA composition and additional hydrophilic regions compared with bovine caseins. Elephant milk is destabilized by 62% alcohol, and it is speculated that the β-casein characteristics may allow maintenance of the colloidal nature of the casein micelle, a role that was previously only associated with κ-casein. The oligosaccharide content of milk was reported to be low in dairy animals but high in some other species such as humans and elephants. In the milk of the African elephant, lactose and oligosaccharides both occur at high levels. These levels are typically related to the content of α-LA in the mammary gland and thus point to a specialized carbohydrate synthesis, where the whey protein α-LA plays a role. We report the characterization of African elephant α-LA. Homology modeling of the α-LA showed that it is structurally similar to crystal structures of other mammalian species, which in turn may be an indication that its functional properties, such as lactose synthesis, should not be impaired.

Key words: African elephant, milk, casein, α-lactalbumin, protein structure

INTRODUCTION

Extensive research has been carried out on the milk of economically exploited dairy animals such as cow, goat, sheep, buffalo, and camel. Yet, many properties that are not explicit in the milk of these animals are still not completely understood. Research on milk from nondairy animals, where these properties are explicit, may provide insight.

The first comprehensive study of African elephant (*Loxodonta africana*) milk was conducted by McCullagh and Widdowson (1970). Later studies by Osthoff and coworkers provided detailed information of protein, fat, and sugar composition (Osthoff et al., 2005, 2007). It was observed that the milk of this species had some unique characteristics.

Milk of most mammalian species contains α_{S1}-, α_{S2}-, κ-, and β-CN, which exist as colloidal aggregates of CN with calcium and phosphate, known as the CN micelle (Walstra, 1999; Farrell et al., 2004). Micelles are responsible for converting milk into a free-flowing, low-viscosity liquid and also provide the means for transportation of high levels of calcium and phosphate in the mammary gland. The CN have AA sequences and structural and functional properties that are different from each other. This is a consequence of different levels of posttranslational modifications such as phosphorylation of Ser (or Thr) and glycosylation of Thr residues, mutational changes in CN genes, proteolysis by indigenous milk proteases, or oxidation of Cys (Swaisgood, 1993; Martin et al., 2003). A great deal of research has gone into unraveling the structure of CN and the CN micelles. However, the greatest challenge remains that CN cannot be crystallized for x-ray crystallographic analysis, nor can they be accurately studied by nuclear magnetic resonance spectroscopy (Farrell et al., 2004). Other methods of micelle structure elucidation, such

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as small-angle x-ray scattering and small-angle neutron scattering, have various limitations (Holt et al., 2013). Consequently, several models have been developed in an effort to describe the CN micelle structure. The most accepted model suggests a cross-linking of CN through hydrophobic regions of the CN and bridging by calcium phosphate nanoclusters, and that stability is maintained by a localized excess of hydrophobic attraction over electrostatic repulsion (De Kruif, 1999; Horne, 2006).

Although the micelle models best describe micelle structure in bovine milk, it must also be taken into account that the amounts of κ- and β-CN vary among species (Horne, 2008). Bovine milk generally has high amounts of κ-CN, whereas milk of some nonbovine species, for example horse, human, and rat milk, contains low amounts of κ-CN relative to total CN, approximately 1:7 and 1:5 for horse and human κ-CN, respectively (Martin et al., 2003). This variability is also evident in human milk, which is one of the most thoroughly studied milks and in which α_{S2}-CN has not been detected (Martin et al., 2003). Moreover, sheep milk also contains 2 nonallelic forms of α_{S2}-CN because of an internal deletion of 9 AA residues (Boisnard et al., 1991).

The relative composition of the CN proteins in the CN micelle also seems to play a role in its stability. Heat and ethanol stability has been studied for milk from several species, and it seems as if the α_{S1}- and κ-CN play the major role. Cow milk contains the highest amounts of these 2 and has the highest stability, compared with other species (Unnikrishnan et al., 1988; Horne and Muir, 1990; Guo et al., 1998; Horne, 2008; Fava et al., 2014).

Results of the current research show that African elephant milk contains high amounts of β-CN and low amounts of κ-CN. However, despite the low amounts of κ-CN, and like all mammalian milks (Martin et al., 2003), African elephant milk still contains CN micelles. This observation prompted an investigation that focused on the characterization of the CN of this species.

It was apparent from previous research that lactose and oligosaccharides in African elephant milk both occur at high levels (Osthoff et al., 2005, 2007). This is unusual, because in general, most eutherian species contain lactose as the dominant milk carbohydrate, whereas monotremes, marsupials, and a few other eutherian species contain oligosaccharides as the major carbohydrate (Urashima et al., 2005).

The synthesis of lactose in the mammary tissue is regulated by α-LA (Klee and Klee, 1970). However, in the absence of α-LA, β-1,4-galactosyltransferase 1 (**β-1,4-GT1**) catalyzes the transfer of the galactosyl moiety from UDP-galactose to *N*-acetylglucosamine to produce *N*-acetyllactosamine, a primary building block of oligosaccharides. Such absence is observed in Cape fur seal milk because of the lack or low level of α-LA gene expression (Sharp et al., 2008). Consequently, the levels of lactose and oligosaccharides in milk are directly dependent on the amounts of α-LA in the mammary gland (Stacey et al., 1995; Ramakrishnan and Qasba, 2001). Humans possess at least 2 other mammary β-galactosyltransferases that catalyze the synthesis of Gal(β1–3)*N*-acetylglucosamine-R and Gal(β1–4)*N*-acetylglucosamine-R structures (Urashima et al., 2009). The catalytic function of these β-galactosyltransferases is independent of α-LA. Because isoglobotriose [Gal(αl-3)Gal(β1–4)Glc] is the most abundant of the oligosaccharides in Asian and African elephant milk (Uemura et al., 2006; Osthoff et al., 2008), it is probably the β-galactosyltransferase that links galactose via an α l-3 bond to the galactosyl moiety of the lactose, that is in competition with α-LA in its affinity for lactose, which may result in disproportionate synthesis of oligosaccharides or lactose.

Several crystal structures of α-LA from other species have been solved, namely human, goat, baboon, guinea pig, cow, and mouse (Pike et al., 1996). However, these species all produce milk that contains low levels of oligosaccharides and high levels of lactose in their milk (Urashima et al., 2009). Because of the low amounts of African elephant milk available and the difficulty experienced with protein crystallization, we followed the route to identify the presence of α-LA in African elephant milk by electrophoresis, determine the sequence by mass spectrometry (**MS**) and databases, and predict the structure of α-LA with homology modeling.

MATERIALS AND METHODS

Sample Preparation

Milk samples were collected from 3 African elephants at different stages of lactation. The early-lactation sample was obtained from Bloemfontein Zoological Gardens, South Africa, 4 d after parturition (Osthoff et al., 2005). The other 2 samples were obtained from Welgevonden private game reserve, Vaalwater, South Africa, at 12 and 18 mo of lactation (Osthoff et al., 2007). Protein precipitation was done with acetic acid and trichloroacetic acid according to the method by Igarashi (1995), and the final protein concentration of the samples was determined by the Bradford assay (Bradford, 1976).

Ethanol Stability

A down-scaled procedure of the ethanol stability test (Fava et al., 2014) was performed by mixing 200 μL