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Amino acid enrichment and compositional changes among mammalian milk proteins and the resulting nutritional consequences

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ABSTRACT

Milk is a hallmark of mammalian evolution: a unique food that has evolved with mammals. Despite the importance of this food, it is not known if variation in AA composition between different species is important to milk proteins or how it might affect the nutritional value of milk. As milk is the only food source for newborn mammals, it has long been speculated that milk proteins should be enriched in essential AA. However, no systematic analysis supports this assumption. Although many factors influence the overall nutritional value of milk, including total protein concentration, we focused here on the AA composition of milk proteins and investigated the possibility that selection drives compositional changes. We identified 9 major milk proteins present in 13 mammalian species and compared them with a large group of nonmilk proteins. Our results indicate heterogeneity in the AA composition of milk proteins, showing significant enrichment and depletion of certain AA in milk-specific proteins. Although high levels of particular AA appear to be consistently maintained, orthologous milk proteins display significant differences in AA composition across species, most notably among the caseins. Interspecies variation of milk composition is thought to be indicative of nutritional optimization to the requirements of the species. In accordance with this, our observations indicate that milk proteins may have adapted to the species-specific nutritional needs of the neonate.
Key words: amino acid composition, amino acid enrichment, milk protein evolution, milk nutrition

INTRODUCTION

Milk, secreted by the mammary glands of females, is a hallmark of mammalian evolution. Known benefits of milk include its rich macro- and micronutrient content and various health-promoting roles. Milk is primarily composed of 3 parts: the protein-rich whey, the casein micelles that transport calcium and give milk its white color, and the milk fat globule (Jensen, 1995). Milk secretion has been conserved for approximately 218 million years (Meredith et al., 2011), from platypus to modern human, as a means of nutrition for the newborn.

Five small milk proteins are strikingly “milk specific”: the whey protein α-LA and the 4 caseins (α_{S1}-CN, α_{S2}-CN, β-CN, and κ-CN), which are synthesized in the mammary gland during pregnancy or at the onset of lactation (Burditt et al., 1981). These proteins are recognized as being the most abundant proteins in the milk of many species, including human and bovine milks (Lemay et al., 2009). Milk also contains many other proteins at lower frequency, such as lactoferrin, which is associated with whey, and mucin, lactadherin, butyrophilin, and xanthine dehydrogenase, all of which are associated with the milk fat globule (**MFG**). These 5 larger milk proteins are not specific to milk, belonging to families of proteins that can also be found outside the mammary gland and whose synthesis is not dependent on pregnancy and lactation (Zotter et al., 1988; Parry et al., 1992; Valenti and Antonini, 2005; Véron et al., 2005). Nine of these relatively abundant milk proteins are present in at least 9 of 13 representative species across Mammalia for which genome sequences are available (i.e., human, chimp, monkey, mouse, rat, guinea pig, rabbit, cat, dog, horse, cow, opossum, and platypus; see Table 1).

Mammals differ greatly in the nutritional contents of their milks. For example, the composition and, in particular, protein concentration of milk varies greatly among species. Of those species extensively studied to date, an interspecies difference in protein concentration ranges from approximately 1 to 20%, and is believed to reflect species-specific optimization to the nutritional requirements of the neonate (Jenness, 1974b; Hambræus and Lönnerdal, 2003; Martin et al., 2013). Further important variation is seen with respect to whey protein-to-casein ratios in milks. In humans, the

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typical whey-to-casein ratio during lactation is approximately 60:40, but can fluctuate from 80:20 to 50:50 during early and late stages of lactation, respectively (Lönnerdal, 2003). However, in the majority of mammalian milks, the converse of this is observed, with the total proportion of casein summing to more than that of whey (Jenness, 1974a, 1986).

Within the caseins alone, which are important transporters of calcium phosphate, significant disparity can be observed among species. For instance, human milk contains only 3 caseins, with bovine and murine milks containing 4 and 5, respectively (Bevilacqua et al., 2006). Moreover, the individual proportion of each casein protein contrasts considerably in mammals. In human milk, β-CN is the principal component of the casein micelle, whereas in rabbits, α_{S1}-CN dominates, with equivalent ratios of both caseins found in murine, porcine, and bovine milks (Martin et al., 2003). It is thus clear that the species-specific adaptation of milk to the nutritional demands of their young is a multifactorial process.

The role of AA composition of milk proteins in conferring optimized nourishment across mammals is less established. It is commonly believed that proteins from the mother's milk meet the AA needs of the newborn, especially the requirement of EAA not naturally produced by mammals (e.g., for human: His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val; and in the case of premature infants, Cys and Tyr; Reeds, 2000; Fürst and Stehle, 2004). This assumption is supported by the fact that milk constitutes the only food source for mammalian neonates and thus should provide the essentials for growth and development beyond those stores established by the offspring in utero.

Food composition and nutrition tables established by the International Network of Food Data Systems (INFOODS), which is part of the Food and Agriculture Organization (FAO) of the United Nations (http://www.fao.org), show that milk-specific proteins, especially whey proteins, are rich in infant EAA compared with other food sources. For example, the whey protein α-LA is shown to be rich in Thr, a precursor of serotonin, which is used as a supplement to relieve sleep disorders (Fernstrom, 1983; Schaechter and Wurtman, 1990). Further to this, whey proteins have higher frequencies of branched-chain AA (Val, Ile, and Leu) than many other food products (Davis et al., 1994; Hui et al., 2007). As a result, whey is used as a supplement for active individuals such as athletes (for a review, see Ha and Zemel, 2003). Surprisingly, to date it has not been shown if certain AA are enriched in milk proteins compared with the rest of the proteome.

Because mammalian species differ in many morphological, physiological, and behavioral traits, we set

Table 1. Presence of the major milk proteins in 13 mammalian genomes[1]

Protein and gene name	Human	Chimp	Monkey	Mouse	Rat	Guinea pig	Rabbit	Cow	Horse	Dog	Cat	Opossum	Platypus
α_{S1}-CN (*CSN1S1*)	+	+	−	+	+	+	+	+	+	+	−	−	−
β-CN (*CSN2*)	+	+	+	+	+	−	+	+	+	+	−	−	+
κ-CN (*CSN3*)	+	+	+	+	+	+	+	+	+	+	−	−	+
α-LA	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactoferrin (*LTF*)	+	+	+	+	+[2]	−	−	+	+	+	−	−	−
Lactadherin (*MFGE8*)	+	+	−	+	+	+	+	+	+	+	−	−	−
Mucin (*Muc1*)	+	+	+	+	+	−	+	+	−	+	−	+	−
Butyrophilin (*BTN1A1*)	+	+	+	+	+	−	+	+	+	−	−	−	−
Xanthine dehydrogenase (*XDH*)	+	+	+	+	+	+	+	+	+	+	+	−	−

[1]All orthologs might not have been detected in our study because of very divergent sequences hampering any similarity-based search methods (see Methods). The presence of a single-copy ortholog is shown by the "+" symbol, whereas absence of a detected ortholog is shown by "−".

[2]Lactoferrin is present in rat but is not found in the milk of this species. See Materials and Methods.