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Research review paper

Recombinant human lactoferrin: A valuable protein for pharmaceutical products and functional foods

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ABSTRACT

Lactoferrin, the main iron-binding protein of milk, has biological activities that are essential for the newborn and are beneficial for adults. Given this beneficial effect, there is broad interest in exogenous sources of lactoferrin in human nutrition. Consequently, several transgenic approaches to produce lactoferrin have been achieved. However, the activity of heterologous lactoferrin cannot be assumed to identically mimic that of the homologous protein. Human lactoferrin obtained from yeast, transgenic cows, and rice has met the criteria of structural similarity, high yield, and ease of protein isolation. Human lactoferrin from *Aspergillus awamori* has been mainly directed to therapeutic uses with advanced phases of clinical trials currently in progress. In contrast, human lactoferrin produced in transgenic cows and rice brings the clear advantage of origins compatible with use in foods, although the approval for these applications is still in process.

Contents

1. Introduction

Milk can be considered the first functional food in life as it contains, not only nutrients for the newborn, but also essential components for organ development and normal physiology. An overarching goal is to identify and produce beneficial components of milk for improving nutrition and for specific therapeutic interventions. While some components are already being isolated from bovine milk to meet this goal, others, especially components of human milk cannot be easily obtained from the natural source. This is illustrated by lactoferrin, a multifunctional protein commonly isolated from bovine milk and whey, which has been the subject of many studies to determine its functional equivalency to the native human protein.

Lactoferrin is a glycoprotein which belongs to the family of non-haem iron-binding proteins which also includes transferrin, ovotransferrin and melanotransferrin (Ward et al., 1996). These proteins are able to bind, in a reversible form, two atoms of ferric iron in the presence of carbonate or bicarbonate ions (Anderson et al., 1987, 1989). All iron-binding proteins present a reddish colour when saturated with iron, yielding a maximum of absorbance between 460 and 470 nm and having a more compact conformation than the iron-depleted form (Anderson et al., 1989).

Sørensen and Sørensen (1939) identified lactoferrin in bovine milk in 1939 and it was subsequently identified in human milk (Schafer,

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1951). The isolation of lactoferrin was achieved simultaneously from bovine and human milk by several research groups (Groves, 1960; Montreuil and Mullet, 1960; Johanson, 1960). Lactoferrin, initially described as the "red milk protein" for its colour upon iron saturation, was designated as lactotransferrin due to homology with transferrin, the serum iron transporter protein, but was later renamed as lactoferrin (LF).

Although lactoferrin was initially thought to be secreted exclusively into milk, it was subsequently identified in other bodily secretions, including saliva, tears, bile, pancreatic fluid, semen, and mucus of the bronchial, nasal, and cervical tracts (Levay and Viljoen, 1995; Weinberg, 2003). Consequently, lactoferrin was then considered to be an exclusive product of epithelial secretion; however, lactoferrin was also found to be a constitutive component of neutrophils, representing the most important source in the blood. Furthermore, other tissues such as the bone marrow, via activity of neutrophil precursors, and placenta have been confirmed as sources of lactoferrin (Levay and Viljoen, 1995).

Comparison of lactoferrin and transferrin reveals both similarities, most notably iron binding and approximate molecular mass, as well as differences. Unlike transferrin, lactoferrin is a basic protein with an isoelectric point of 8.4–9.0 (Moguilevsky et al., 1985) and, despite sequence homology, there is no immunological cross-reaction between the two. Moreover, the affinity constant of lactoferrin for iron is 300 times greater than that of transferrin (Aisen and Leibman, 1972) and in the presence of citrate ion, lactoferrin can retain the iron atom down to pH 2, while transferrin loses it at pH 5 (Mazurier et al., 1983).

Lactoferrin has been detected in numerous mammalian species, milk being the most abundant source of this protein in all of them (Farnaud and Evans, 2005). Consistent with sequence homology, the internal structure of lactoferrin is highly conserved among the different species with capture of the iron atoms in two nearly identical sites, one in each lobe of the molecule. The process of iron binding and release and the conformational changes which take place in the lactoferrin molecule during this process have been extensively studied (Fig. 1) (Anderson et al., 1989; Anderson et al., 1990; Baker et al., 1991; Baker and Baker, 2005, 2009). The main functional properties of lactoferrin are derived from this property of iron scavenging and the dynamic changes in the protein structure reflects its iron status. In contrast to the highly conserved internal structure of lactoferrin among mammalian species, the external structure of lactoferrin is much more variable making it more difficult to identify relevant sites for function apart from iron binding (Baker and Baker, 2009). All lactoferrins characterized to date are cationic, a property that is a primary determinant of the ability to bind to different cell types and to anionic molecules. The positive region is located in the N-terminal domain and several functional, cationic peptides, such as lactoferricin and lactoferrampin, are derived from the N-terminal domain via hydrolysis. Correspondingly, the basic residues in the intact protein and the peptides have been proposed to be responsible for the anti-bacterial properties of lactoferrin via disruption of bacterial cell membranes (Baker and Baker, 2005). All lactoferrins characterized to date are glycosylated, however the number and location of potential and actual glycosylation sites vary from protein to protein (Baker and Baker, 2009).

Lactoferrin has been proposed to be involved in several biological functions, such as regulation of iron transport, anti-microbial defense, anti-tumour mechanisms, and immune system regulation (Brock, 2002; Ward et al., 2005). Recently, oral administration of lactoferrin has been reported to benefit human and animal health, due to these anti-microbial, anti-carcinogenic and anti-inflammatory activities (Wakabayashi et al., 2006). These findings support the utility of lactoferrin as an ingredient in foods and special products (Tomita et al., 2009). Large-scale manufacturing of bovine lactoferrin (bLF) was established more than 20 years ago, using bovine skim milk and whey as sources. Oleofina Company in Belgium and MILEI GmbH in Germany (using technology developed by Morinaga Milk Industry Co., Ltd.) pioneered industrial scale bLF production (Tomita et al., 2009). Currently, bLF is added as a supplement to several products in Japan, including infant formula, yogurt, specialized milk-based and other beverages, nutritional supplements, pet foods, and cosmetics (Wakabayashi et al., 2006). Similarly, infant formulas enriched with bLF are also available in other countries, including Indonesia, South Korea, and Spain. However, whether the activity of bovine lactoferrin is the same as that of human lactoferrin (hLF) for all proposed

Fig. 1. The figure shows the structure of human lactoferrin obtained from Protein Data Bank entry 1SQY (Vikram et al., in press). Note the two iron atoms (light orange) bound together with two carbonate ions (white and red) to each lobe of the protein.