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Purification of Lactoferrin from Camel colostrum and Protein Profiles of Camel and Bovine Milk

Fawzi O. Ebrahim[1,*], Abdul-munam M. Fellah[1], Saber Eldarhobi[1], Adam Elzagheid[1]
[1]Biotechnology Research Center (BTRC), Libya

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***Corresponding to:**
fowzi.omar@yahoo.com

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ABSTRACT

Human and bovine lactoferrin have been studied extensively, but very few reports exist on camel (*Camelus dromedarius*) lactoferrin. This iron-binding glycoprotein is present primarily in milk. It has been shown to be involved in various physiological and protective functions, including homeostasis and cell proliferation, and it has antibacterial, antifungal, antiviral, antioxidant, immunomodulatory and anticancer activities. This study aimed to compare the protein profiles of camel milk and bovine milk by gel electrophoresis and to isolate camel lactoferrin from colostrum. Camel milk proteins profile lacked β-lactoglobulin. Lactoferrin was isolated from colostrum by cation exchange chromatography and identified by its molecular weight after gel electrophoresis as a single band of about 78 kDa, demonstrating the purity of the isolated protein. The study demonstrates a simple one-step method to purify lactoferrin from camel colostrum.

1. INTRODUCTION

Milk is an important nutrition source for people around the world. Recently, much consideration has been given to milk quality, especially milk protein (Shi, et al., 2010). The two most abundant proteins in milk are caseins (insoluble) and whey proteins (soluble). Several studies focused on characterization of bovine whey proteins using proteomic approaches (Manso, et al., 2005). The major bovine whey protein fractions are α-lactalbumin (α-LA, 14 kDa) and β-lactoglobulin (β-LG,17 kDa). Bovine whey also contains several minor but extensively studied proteins, such as lactoferrin (Lf; 76–78 kDa) (Sèverin and Wenshui, 2005). Colostrum is the early milk produced during the first several days after parturition and its composition is different from that of the milk produced later. Colostrum is not only a source of nutrients such as proteins, carbohydrates, fat, vitamins and minerals but also contains several biological molecules that are essential for specific functions, including more immunoglobulins, which provide the newborn with immediate protection and growth factors (Pakkanen and Aalto, 1997; Kelly, 2003). Camel (*Camelus dromedaries*) milk has been reported to possess not only nutritional value but also therapeutic potential for humans (Kumar, et al., 2015). A beneficial role of raw camel milk as a nutritional supplement in chronic pulmonary tuberculosis patients was observed (Mal et al., 2006). Compared to cow milk, camel milk is rich in vitamin C (Asres and Yusuf, 2014), niacin, vitamins A and E, polyunsaturated fatty acids and minerals (sodium, potassium, iron, copper, zinc and magnesium) and poor in cholesterol and lactose (Haddadin, et al., 2008). Like human milk, camel milk has a high content of α-LA and Lf but lacks β-LG (El-Hatmi, et al., 2007). It has no allergenic properties and can be consumed by lactase-deficient and immune-deficient people (El-Agamy, et al., 2009). Lactoferrin (Lf) is an iron binding glycoprotein of 76–78 kDa of the transferrin family widely found in mammalian milk and most other exocrine secretions such as tears, nasal and bronchial mucous, and saliva (Legrand, et al., 2008)). Lactoferrin was isolated for the first time from bovine milk and

later from human milk (Sornesen and Sornesen, 1939). Most of the pioneer research was on human lactoferrin followed by bovine lactoferrin (Baker and Baker, 2005). Lactoferrin has been used in different products, such as infant formulas, probiotics, supplemental tablets, cosmetics and as a natural solubilizers of iron in food (Sreedhara, et al., 2010).

Numerous studies have been conducted on milk from different animals. However, despite the reported benefits and medicinal value of camel milk, it has received little attention (Ereifej, et al., 2011). But recently, the potential therapeutic value of camel milk has received increased attention worldwide.

In this study, we purified lactoferrin from camel colostrum by CEC and investigate the protein profiles of camel milk and bovine milk.

2- MATERIAL AND METHODS

A colostrum samples was obtained immediately after postpartum of a shecamel at a farm in Sirt, Libya and frozen at -20°C. Camel and bovine milk were purchased from a local market in Tripoli city. Camel and bovine milk samples were centrifuged without dilution at 3000 x g at 4°C for 30 min, then yellow fat layer was removed. The frozen colostrum was thawed in its container under running water at room temperature then diluted 1:1 with sterile phosphate-buffered saline pH 7.4 before centrifugation. The yellow fat layer was discarded and the supernatant (whey) was collected and frozen at -20°C (Ebrahim, et al., 2014). For lactoferrin purification, casein was first precipitated from camel whey by adjusting the pH to 4.6 with 1 M HCl and removed by centrifugation at 3500 x g for 30 mins at 4°C. The pH of the supernatant (whey) was adjusted to 7.4 with 1 M NaOH. Globulins and other high molecular weight proteins in the adjusted whey sample were precipitated by 45% saturation with ammonium sulphate and removed by centrifugation. Then whey was filtered through 0.45 mm and 0.22 mm filters. Camel lactoferrin was purified from the whey by cation exchange chromatography on SP-Sepharose as described (Van Berkel, et al., 1995). The column was first packed with food grade SP Sepharose big beads (Amersham Biosciences, 17-0657-03) and washed with 5 column volumes of distilled water followed by 5 column volumes of 1 M NaCl, after which it was left for 12 h before being washed with 100 ml of distilled water. The 10 ml of diluted skimmed colostrum was loaded and the column was washed with 25 ml of a buffer containing 0.02 M NaH_2PO_4, 0.4 M NaCl and 0.02% (v/v) Tween 20, pH 7.4, to remove unbound proteins. Bound protein was eluted with 0.02 M NaH_2PO_4, 1 M NaCl, pH 7.4 at a flow rate of 3 ml/min. The elution fractions were dialysed against sterile milli-Q water. This led to removal of salt as well as all the low molecular weight (15 kDa and below) proteins present in the sample. The elution fractions were kept at 4°C and analyzed in a 12% Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were stained with Coomassie Brilliant Blue R 250, 0.125% (w/v) in 10% acetic acid and 50% methanol for 60 minutes. Destaining was carried out in a solution of 10% acetic acid and 50% methanol in deionized water to identify the relevant fractions.

3. RESULTS AND DISCUSSION

SDS-PAGE was used to compare the milk protein profiles of camel and bovine. Figure 1A shows camel milk lactoferrin at about 78 kDa and same result was found in bovine milk proteins sample figure 1B. The α-casein band was medium, the β-casein band was intense, and the κ-casein band was weaker. The α-LA band was medium. The similar bands dominating the protein profiles of both species were identified as β-casein. α-LA (14 kDa) is seen in the profiles of both species (Fig. 1A, 1B). β-LG (18 kDa) is seen in bovine whey but not in camel whey. This is in agreement with previous findings (Beg, et al., 1989; Farah, 1993). It has been reported that the absence of β-LG in camel milk is important for its properties of preventing and curing food allergies (Merin, et al., 2001; Elhaj and Freigoun, 2015). SDS-PAGE analysis of camel lactoferrin purified by cation exchange chromatography (Figure 2) shows a single band of Camel Lactoferrin (CLf) at ≈ 78 kDa, which could confirm the identity as well as the purity of the purified protein. The presence of band in the eluted fractions at the same position as that of CLf confirmed the identity of the protein as lactoferrin. Absence of any other proteins in these fractions facilitated the confirmative identification of lactoferrin by SDS-PAGE. SDS-PAGE has been used to confirm the molecular weight and purity of lactoferrin (Adam, et al., 2008; Abbas, et al., 2015). CEC on SP-Sepharose considered one of the main technique in terms of isolation Lf (El-gamy, et al., 1996). Moreover, Conesa et al (1982) purified CLf using CEC by SP-sepharose resin from camel milk and from different animal species.

Figure 1: A, Lane 1 marker, other lanes camel milk proteins. B, Bovine milk proteins

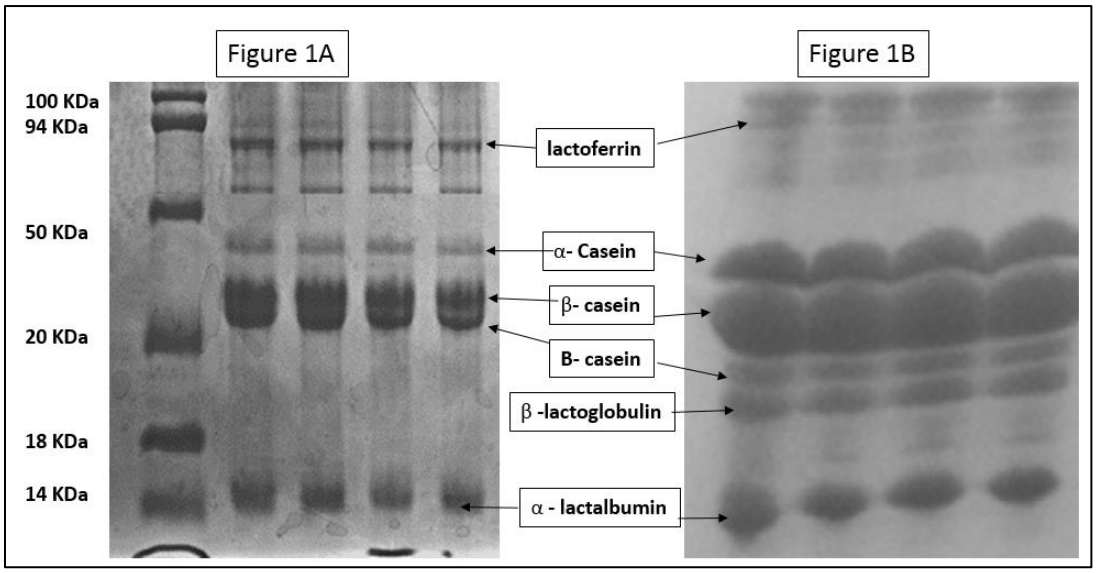
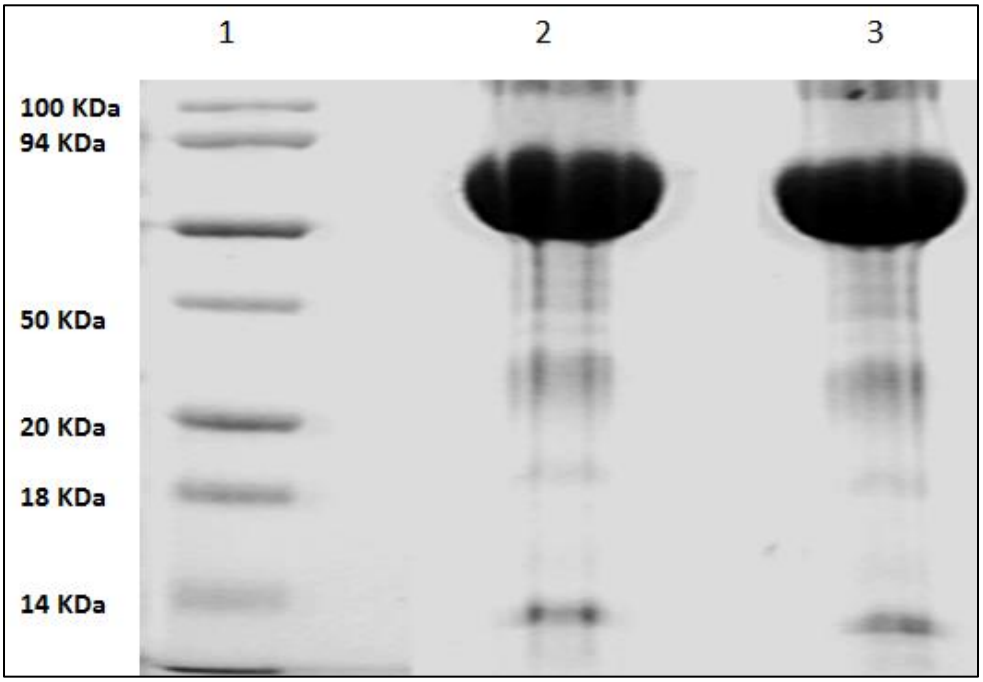


Figure 2: Lane 1 marker, lanes 2 and 3 camel lactoferrin fractions purified from colostrum samples



4. CONCLUSION

The present study show that most camel serum proteins in milk are similar in molecular weight to bovine milk proteins. The main differences between the two species is the absence of β- LG from camel milk proteins. Moreover, camel lactoferrin was successfully purified from camel colostrum by cation exchange chromatography

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6. REFERENCES

Abbas, Z.H., Doosh, K.S., Yaseen, N.Y. 2015. Isolation, purification and characterization of lactoferrin from goat colostrum whey. Pak. J. Nutr.14: 517-523.

Adam, V., Zitka, O., Dolezal, P., Zeman, L., Horna, A., Hubalek, J., Kizek, R. 2008. Lactoferrin isolation using monolithic column coupled with spectrometric or micro-amperometric detector. Sensors. 8(1): 464-487.

Asres, A., Yusuf, M. 2014. Traditional Consumption, Therapeutic Value and Its Derived Dairy Products of Dromedary Camel (Camelus Dromedaries) Milk in Somali Regional State, Eastern Ethiopia: A Review. Glob. J. Anim. Seci. Res. 3(3): 240-246.

Beg, O.U., Von-Bahr-Lindststrom, H., Zaidi, H. 1989. Characterizations of camel milk protein rich proline identify a new beta casein fragment. J. H. Regul. Pept. 15:55-62.

Conesa, C., Sanchez, L., Rota, C., Pérez, M.D., Calvo, M., Farnaud, S., Evans, R.W. 2008. Isolation of Lactoferrin from Milk of Different Species: Calorimetric and Antimicrobial Studies. Comp. Biochem. Physiol. 150(1):131-139.

Ebrahim, F., Shankaranarayanan, J.S., Kanwar, J.R., Gurudevan, S., Krishnan, U.M., Kanwar, R.K. 2014. Identification of unprecedented anticancer properties of high molecular weight biomacromolecular complex containing bovine lactoferrin (HMW-bLf). PLoS One. 9(9): 15.

El-Agamy, E.I., Ruppanner, R., Ismail, A., Champagng, C.P., Assaf, R. 1996. Purification and Characterization of Lctoferrin, Lactoperoxydase, Lysozyme and Immunoglobulins from Camel's Milk. Int. Dairy. J. 6(2):129-145.

El-Agamy, E.I., Nawar, M., Shamsia, S.M., Awad, S., Haenlein, G.F.W. 2009. Are camel milk proteins convenient to the nutrition of cow milk allergic children? Small. Rumin. Res. 82(1*)*:1-6.

El-Hatmi, H., Girardet, J.M., Gaillard, J., Yahyaoui, M.H., Attia, H. 2007. Characterization of whey proteins of camel milk and colostrum. Small. Rumin. Res.70 (2-3): 267-271.

Elhaj, A.E., Freigoun, S. 2015. Camel's Milk Protein Fractionation by SDS-Polyacrylamide Gel Electrophoresis. Int. J. Agro. Vet. Med. Sci. 9(2):41-45.

Ereifej, K., Alu'datt, M., AlKhalidy, H., Alli, I., Rababah, T. 2011. Comparison and Characterization of Fat and Protein Composition for Camel Milk from Eight Jordanian Locations. Food. Chem. 127(1):282-289.

Farah, Z. 1993. Composition and characteristics of camel milk. J. Dairy. Res. 60(4):603-626.

Haddadin, M.S., Gammoh, S.I., Robinson, R.K. 2008. Seasonal variations in the chemical composition of camel milk in Jordan. J. Dairy. Res. 75(1):8-12.

Kelly, G.S. 2003. Bovine colostrum: a review of clinical uses. Altern. Med. Rev. 8(4):378-394.

Kumar, Y.K., Rakesh. K., Lakshmi. P., Jitendra, S. 2015.Composition and medicinal properties of camel milk. A Review. Asian. J. Dairy. Food. Res. 34(2):83-91.

Legrand, D., Pierce, A., Elass, E., Carpentier, M, Mariller, C, Mazurier, J. 2008. Lactoferrin structure and functions. Adv. Exp. Med. Biol. 606:163–194.

Mal, G., Sena, D.S., Jain, V.K., Sahani, M.S.2006 .Therapeutic value of camel milk as a nutritional supplement for multiple drug resistant (MDR) tuberculosis patients. J. Vet. Med. 61: 88-91.

Manso, M.A., Leonil, J., Jan, G., Gagnaire, V. 2005. Application of proteomics to the characterisation of milk anddairy products. Int. Dairy. J. 15: (6-9): 845-855.

Merin, U., Bernstein, S.D., Bloch-Damti, N., Yagil, R., van Creveld, C., Lindner, P. 2001. A comparative study of milk proteins in camel (Camelusdromedarius) and bovine colostrum. Livestock. Prod. Sci. 67(3):297-301.

Pakkanen, R., Aalto, J. 1997. Growth factors and antimicrobial factors of bovine colostrum. Int. Dairy. J. 1997. 7(5):285-297.

Séverin, S., Wenshui, X. 2005. Milk biologically active components as nutraceuticals: Review. Criti. Revue Food. Sci. 45(7-8):645-656.

Sreedhara, A., Flengsrud, M., Langsrud, R. 2010. Structural characteristic, pH and thermal stabilities of apo and holo forms of caprine and bovine lactoferrins. Biometals. J. 23(6):1159-1170.

Sorensen, M., Sorensen, S.P.L. 1939. The protein in whey. Comptes-rendus Des Travaux Du Laboratoire Carlsberg. 23(7): 55-99.

Van Berkel, P.H.C., Geerts, M.E.J., van Veen, H.A., Kooiman, P.M., Pieper, H.A., de Boer, A., Nuijens, J.H. 1995. Glycosylated and unglycosylated human lactoferrin both bind iron and show identical affinities towards human lysozyme and bacterial lipopolysaccharide, but differ in their susceptibilities towards tryptic proteolysis. Biochem. J. 312(1):107–114.