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Finnish and Norwegian reindeer[1] milk betalactoglobulin; characterization of genetic variants[2]

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Introduction

Betalactoglobulin (βLG) is the main whey protein in most ruminants and belongs to the lipocalin protein family (Flower, 1996). βLGs from different ruminant species share over 90% sequence homology. The homology in nonruminants' monomeric βLGs is only about 30% to 70%. βLG does not appear in human and rodent milk (Hambling *et al.*, 1992). According to previous data altogether 12 variants are expressed in bovine βLG from which variants A and B are predominant and most common (Hambling *et al.*, 1992). The isoelectric points of bovine milk βLGs are 5.1 (A) and 5.3 (B) (Rytkönen *et al.*, 2002) and the molecular mass of the monomers about 18.000. Chemical and physical properties of bovine milk βLG are known while its biological function and its role as a transport protein are yet unclear. According to previous data when βLG was isolated from the milk of seven Finnish semidomestic reindeer, βLG has only one genetic variant the IP (isoelectric point) of which is about 4.9 (Rytkönen *et al.*, 2002; Heikura *et al.*, 2005). For red deer an earlier report indicates the IP about 5.17 (McDougall & Stewart, 1976).

Here our aim was to characterize Finnish (eight) and Norwegian (ten) reindeer milk βLG proteins by using very sensitive electrophoretic methods, and compare the results with earlier data obtained by using less sensitive methods. The reindeer milk βLG variants were also compared with those of bovine milk βLG.

Materials and Methods

Finnish reindeer milk were obtained from the Reindeer Research Station, Kaamanen, Norwegian reindeer milk from the Norwegian University of life Sciences (Department of Animal and Aquacultural Sciences, Ås, Norway) and bovine milk from a local farmer in Sotkamo, Finland. βLG proteins were isolated as described earlier (de Jongh *et al.*, 2001; Heikura *et al.*, 2005). βLGs were analyzed by Native-PAGE (Heikura *et al.*, 2005), by reduced SDS-PAGE (Heikura *et al.*, 2005), by IEF (Heikura *et al.*, 2005) and by Western blotting (Rytkönen *et al.*, 2002). Electrofocusing was done using a Phast apparatus and pH gradient gels (Phast Gel IEF 3-9 or 4-6.5; Rytkönen *et al.*, 2002; Heikura *et al.*, 2005). Polyclonal antibody produced in rabbits to native bovine milk βLG was used as the primary antiserum.

Results

The molecular masses of the milk proteins were evaluated by reduced SDS-PAGE followed by Western blotting with identification of the βLG proteins by polyclonal antisera to bovine milk βLG. Our data indicates that both Finnish and Norwegian reindeer milk βLG proteins as well as bovine milk βLG proteins showed a similar molecular mass as estimated by a reduced SDS-PAGE.

All βLG proteins were analyzed also by native PAGE in long gels (20 cm) to study their charge differences and molecular masses. βLG proteins were identified by Western blotting; The antisera to bovine milk βLG recognised two protein bands in all milk samples but the mobilities of the two βLG

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[2] Abstract in Finnish entitled Poronmaidon betalaktoglobuliini: geneettiset varantit, see *Rangifer* Report No. 11 (2006): 74.

bands in bovine milk were different as compared to those in reindeer milk. In addition in reindeer milk the mobility and intensity of the other βLG band varied and was different compared to that of bovine milk βLG.

In the present study charge differences of the βLG bands were studied also by electrofocusing in narrow pH gradients followed by Western blotting and showed two βLG bands that were recognised by the antisera to bovine milk βLG. The isoelectric points of the βLG bands in reindeer milk were about 4.8 and 4.9, and differed when compared to those in bovine milk (IP 5.1 and 5.3) in accordance with the data obtained with native PAGE as described above. In addition the IEF gel stained with CBB R-250 showed that some Norwegian reindeer milk βLG variants with IPs about 4.9 consisted of two subvariants.

Conclusions

Genetic variants of βLGs from Finnish and Norwegian reindeer milk (18 reindeer) were studied and βLG isolated from bovine milk was used as a control. All βLGs were recognized by polyclonal antisera to bovine milk βLG and showed a similar molecular mass as analyzed by a reduced SDS-PAGE.

All βLGs showed two genetic variants when analyzed by native PAGE in long gels. The molecular mass of the Finnish and Norwegian reindeer milk βLG variants were similar, but differed when compared to those two variants of bovine milk βLG. This indicates that since the molecular masses were similar, the differences in the βLG proteins are charge ones.

Charge differences were studied further by electrofocusing in narrow and wide pH gradients. All βLGs appeared as two main variants, but were different in reindeer milk (IPs approximately 4.8 - 4.9) compared to those in bovine milks (IPs 5.1 - 5.3). In addition in reindeer milk, the protein band with IP about 4.9 appeared as two bands in most reindeer milk. Further studies such as determination of amino acid composition and sequencing are needed to clarify in details the genetic variants of Finnish and Norwegian reindeer milk βLGs.

References

de Jongh, H. H. J., Gröneveld, T. & de Groot, J. J. 2001. Mild isolation procedure discloses new protein structural properties of β-lactoglobulin. – *J. Dairy Sci.* 84 (3): 562-571.

Flower, D. R. 1996. The lipocalin protein family: structure and function. – *Biochem. J.* 318: 114.

Hambling, S. G., McAlpine, A. S. & Sawyer, L. 1992. B-lactoglobulin. – *In*: Fox, P. F. (ed.). *Advanced Dairy Chemistry. Vol 1. Proteins.* Elsevier Applied Science: London and New York, pp. 141-190.

Heikura J., Suutari T., Rytkönen J., Nieminen M., Virtanen V. & Valkonen K. 2005. A new procedure to isolate native β-lactoglobulin from reindeer milk. – *Milchwissenschaft* 60 (4): 388-392.

McDougall, E. I. & Stewart, J. C. 1976. The whey proteins of the milk of red deer (*Cervus elaphus* L.). A homologue of bovine beta-lactoglobulin. – *Biochem. J.* 153: 647-655.

Rytkönen, J., Alatossava, T., Nieminen, M. & Valkonen, K. 2002. Isolation and characterization of b-lactoglobulin from reindeer milk. – *Milchwissenschaft* 57: 259-261.

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