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研究ノート

Relationships between polymorphic variants and expression level of the corresponding constituents in major milk protein loci of Holstein-Friesian cows

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Abstract

The relationship between polymorphic variants of major milk protein〔α_{S1}-casein (Cn), β-Cn, κ-Cn and β-lactoglobulin (Lg)〕and expression quantity of the corresponding constituents in Holstein-Friesian cows was examined to obtain information on the relevance of protein polymorphisms to functionality.

In an ELISA (sandwich method) using purified monoclonal antibody (mAb), detection sensitivity improved to about 10^2 to 10^5 fold compared to the solid phase method which was previously reported for determining the quantity of α_{S1}-Cn, β-Cn, κ-Cn and β-Lg per 1 ml of skim milk. As a result of comparing the expression quantity (A_{405}) of a component with the corresponding variants within four major milk protein loci, individuals with κ-Cn·B/B type and β-Lg·A/A type were found to have excellent expression quantity for κ-Cn and β-Lg constituents, respectively. Though for the β-Cn type, the result differed from that obtained with the solid phase method, the result which A^2/A^2 type produced more β-Cn quantity than A^1/A^1 type was the same as the previous one. As an example of bovine milk protein polymorphism, it was observed that the expression quantity of constituents was significantly different among polymorphic variants within the corresponding locus.

Introduction

Protein markers in domestic animals have mainly been applied to pedigree registration and the phylogeny of livestock together with blood group factors. Though economically useful, such information is of little value. Linkage among the porcine halothane sensitivity (*Hal*) locus, phosphohexose isomerase (*PHI*) locus and 6-phosphogluconate dehydrogenase (*6-PGD*) locus, and the association between cattle milk protein variants and milk yields or milk fat, etc. was clarified (Rasmusen *et al.* 1980, Komatsu *et al.* 1981, Ng-Kwai-Hang *et al.* 1984, 1986, 1990).

Protein variants are electrophoretically detected based on differences in molecular size and microheterogeneity which are caused by glycosylation of the protein molecule and conformation of the polypeptide chain etc.. The association between many protein variants and physiological functions have not been precisely explained. And relationship between polymorphic variants and the expression level of the corresponding constituents has not yet been reported.

Accordingly, for the dairy milk protein constituent, the relationship between genetic variants and expression quantity of the corresponding constituents was examined to elucidate relevance of polymorphism to functionality.

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Materials and methods

1. Cow milk samples

811 milk samples from Holstein-Friesian cows were used for typing and the estimation of the amount of protein components. The samples were mainly collected from 15 farms in the Ubaranai region of Abashiri City, Hokkaido. Milk fat and admixtures were removed by centrifugal separation (3000 rpm, for 20 minutes), and made into skim milk. α_{S1}-casein (Cn), β-Cn, κ-Cn and β-lactoglobulin (Lg) type for all samples were detected by urea-isoelectric focusing (Yokohama and Hirayama

1996). Still, factors such as the breeding conditions, parity, stage of lactation and age were carried out without purposely considering the measurement of each component.

2. Avidin-Biotin system ELISA (Sandwich method)

Blood was sampled after the milk protein sample (α_{S1}-Cn; C7891, β-Cn; C6905 κ-Cn; C0406 and β-Lg; L6879, SIGMA) was injected into a rabbit intracutaneously. Then anti-cattle milk protein polyclonal antibodies obtained by ammonium sulfate precipitation were coated in the solid phase in microtiter plates as a primary antibody, and milk protein antigen was added. Next, secondary antibody labeled with biotin after being purified from serum-free culture filtrate of α_{S1}-Cn (two cell lines), β-Cn (five cell lines), κ-Cn (three cell lines) and β-Lg (two cell lines) of anti-cattle milk protein monoclonal antibodies (mAbs) which we produced (Yokohama *et al.* 1996), reacted to the antigen combined on the plate. Afterwards, the antigen level was measured based on absorbance (A_{405}) using the sandwich method applied Avidin-Biotin system (Javois L. C. 1999). Still, the pH of the Tris-HCl buffer for washing and reagent dilution was the optimum for each mAb in the antigen-antibody reaction as reported by Watanabe *et al.* (1999). Preparation of a standard curve was carried out by the method of Hirayama and Yokohama (1997), and was used to estimate the average content per 1ml of the major four milk components. However, relation between polymorphism and milk component amounts were analyzed using the absorbance value (A_{405}). The significance of the expression quantity was analyzed by method of analysis of variance.

Results and discussion

The average amounts (mg/ml±S.E.) of major cattle milk components (α_{S1}-Cn, β-Cn, κ-Cn and β-Lg) calculated from the standard curve for each was $(13.53\pm1.89)\times10^{-1}$, $(6.42\pm1.65)\times10^{-1}$, $(0.31\pm1.18)\times10^{-2}$ and $(1.37\pm4.78)\times10^{-2}$, respectively. The detection limit was therefore about 10^2 to 10^5 fold lower than that of the ELISA solid phase method reported by Hirayama and Yokohama (1997) as shown in Table 1. As we used the primary purified polyclonal antibody and purified mAb from the culture supernatant, the detection sensitivity might be improved. However, in order to determine protein quantity, the calibration curve must be reset for every measurerment. The absorbance value (A_{405}) was used as the quantitative value of polymorphic variant in order to measure multispecimens to save on cost and time. As a result of comparing expression levels between each variant of the milk component based on the absorbance value (Table 2), in the α_{S1}-Cn type, even though there was no difference in α_{S1}-Cn amount between B/B and B/C types, the former tended to have a slightly higher output. However, the B/B type had a significantly high α_{S1}-Cn amount in a previous results (Hirayama and Yokohama 1997). Though the β-Cn·A^2/A^2 type had the highest β-Cn amount in the previous report, the β-Cn quantity of A^1/B type was the highest in the present study, followed by the A^2/A^2 type in experiments used over 20 samples. There was a significant difference between dimorphous. In A^1/A^1, A^1/A^2 and A^2/A^2 types, for which many examples could be investigated, A^2/A^2 type had a higher β-Cn amount than A^1/A^1 and A^1/A^2 types.

κ-Cn·B/B type had the most κ-Cn as in the previous study, followed by A/B type. B/B type had the highest output (Hirayama and Yokohama 1997). Since there was a report that the κ-Cn·B/B type individuals had higher milk yields and milk protein productivity (Komatsu *et al.* 1981, Ng-Kwai-Hang *et al.* 1984), it seemed that κ-Cn productivity may be improved by increasing the B/B type individuals in the Holstein-friesian popula-

Table 1 Detection limit between sandwich method and solid phase method

Milk proteins	Sandwich method	Solid phase method ※
α_{S1}-Cn	$(13.532\pm1.89)\times10^{-1}$	$(1.573\pm8.45)\times10^{-2}$
β-Cn	$(6.420\pm1.65)\times10^{-1}$	$(0.019\pm3.54)\times10^{-4}$
κ-Cn	$(0.312\pm1.18)\times10^{-2}$	$(0.003\pm7.80)\times10^{-5}$
β-Lg	$(1.369\pm4.78)\times10^{-2}$	$(0.001\pm3.83)\times10^{-5}$

Cn: Casein, Lg: Lactoglobulin
Figures: Average ±S.E. (mg/ml)
※: by Hirayama H. and M. Yokohama (1997)

Table 2 Relationship between polymorphic variants and expression level of the corresponding bovine milk components

α_{s1}-Cn type	B/B	B/C	Average
Number(%)	792 (97.7)	19 (2.3)	811
α_{s1}-Cn (Average±S.E.)	$(0.230\pm0.2)\times10^{-2}$	$(0.228\pm0.9)\times10^{-2}$	$(0.230\pm0.2)\times10^{-2}$

β-Cn type	A^1/A^1	A^1/A^2	A^2/A^2	A^1/B	A^2/B	B/B	$A^3/?$	Average
Number (%)	116 (14.3)	374 (46.1)	249 (30.7)	25 (3.1)	38 (4.7)	1 (0.1)	8 (1.0)	811
β-Cn (Average±S.E.)	$(0.135\pm0.5)\times10^{-2b}$	$(0.141\pm0.3)\times10^{-2b}$	$(0.142\pm0.4)\times10^{-2b}$	$(0.172\pm1.5)\times10^{-2a}$	$(0.139\pm0.8)\times10^{-2b}$	0.169	$(0.146\pm2.3)\times10^{-2ab}$	$(0.141\pm0.2)\times10^{-2}$

κ-Cn type	A/A	A/B	B/B	Average
Number (%)	576 (71.0)	225 (27.7)	10 (1.2)	811
κ-Cn (Average±S.E.)	$(0.303\pm1.3)\times10^{-2}$	$(0.346\pm2.1)\times10^{-2}$	$(0.388\pm1.1)\times10^{-2}$	$(0.317\pm1.1)\times10^{-2}$

β-Lg type	A/A	A/B	B/B	Average
Number (%)	84 (10.4)	370 (45.6)	357 (44.0)	811
β-Lg (Average±S.E.)	$(0.836\pm0.6)\times10^{-1a}$	$(0.723\pm2.6)\times10^{-1b}$	$(0.570\pm2.1)\times10^{-1c}$	$(0.667\pm1.7)\times10^{-1}$

A siginificant difference was recognized at a 5 % level between different codes without *β-Cn · B/B type.*
Average value; Each component amount was shown by absorbance(A_{405})
Cn: Casein, Lg: Lactoglobulin

tion. β-Lg·A/A type had more amount of β-Lg component than the other genotypes. A/B type showed a value between those of both homozygotes as well as the κ-Cn type, and there was a statistically significant difference. For the α_{s1}-Cn and β-Cn types, there was different results from that in the previous study (Hirayama and Yokohama 1997). The same results for κ-Cn and β-Lg types as last time indicated that the reliability was high.

In the experiment using multispecimens, it was considered that the superiority of the β-Cn·A^2/A^2 type did not change which was the same as previously. The β-Lg·A/A type individuals were generally predominant. Though it was reported that the A/A type was also predominant in terms of milk protein percentage (Komatsu *et al.* 1981, Ng-Kwai-Hang *et al.* 1984), the frequency of the A/A type individuals were also low (10.4%).

Referring to bovine milk protein polymoryphisms, it was observed that the expression level of each component in milk protein was respectively different among polymorhic variants within the corresponding four major milk protein loci. Though the association between protein polymorphism and economical characteristics had been clarified, this time, the relationship between the polymorphic variants and the expression level of the corresponding protein was clarified by using an immunochemical technique.

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