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Effects of methionine and its ratio to lysine on expression of α_{s1} casein gene in cultured bovine mammary epithelial cells*

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ABSTRACT

Effects of methionine dose and its ratio to lysine on α_{s1} casein gene expression were evaluated in cultured bovine mammary epithelial cells. Methionine was added at levels of 0-100 μg/ml and ratio of lysine to methionine was set at 1-4.5:1. Expression of α_{s1} casein gene was enhanced by addition of methionine up to 60 μg/ml, and then leveled off. There was an increasing trend in α_{s1} casein expression with the increasing ratio of lysine to methionine and the highest level was at 3.5:1. These results indicated that adequate quantities and ratio of amino acids are required to promote milk protein synthesis.

KEY WORDS: mammary epithelial cell, methionine, lysine, α_{s1} casein gene expression

INTRODUCTION

Methionine (Met) and lysine (Lys) are often considered to be co-limiting amino acids (AA) for milk protein synthesis (Schwab et al., 1992). Most of the reported responses in milk and milk protein yields to infused Met and Lys have been in cows given diets prepared to be severely limiting in one, or both, AA. The influence of adequate quantities and ratio of Met and Lys on milk protein synthesis still remains uncertain. A number of models have been developed to examine AA metabolism in the mammary gland. Until recently, there has not been a comprehensive representation of AA metabolism in the udder of the lactating dairy cow (Hanigan et al., 2002). The epithelial cells derived from bovine

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mammary gland are suitable for *in vitro* model to study mammary function (Wu et al., 2004; Zhao et al., 2005). In the present study, we adopted the cells to evaluate the effects of Met and its ratio to Lys on the expression of α_{s1} casein gene.

MATERIAL AND METHODS

Reagents

DMEM/F12 was obtained from Gibco BRL Life Technologies (Carlsbad, CA). Reagents for reverse transcriptase (RT)-PCR assay were purchased from Promega (Madison, WI). Trypsin was obtained from Sigma (Louis, MO). All other reagents were of the highest purity commercially available, purchased from Sangon (Shanghai).

Culture of mammary epithelial cells

Mammary tissues were obtained from two slaughtered Holstein dairy cows at the middle stage of lactation. Tissues were cut into 1 mm^3 pieces and incubated in culture plates (Nunc, Denmark) at 37°C in a water-saturated atmosphere of 95% air and 5% CO_2. The basal medium was supplemented with 2 mM glutamine, 100 IU/ml penicillin and, μg/ml: streptomycin 100, insulin 10, transferrin 5, prolactin 5, hydrocortisone 2 and foetal calf serum (FCS) 10. After the cells covered 80% of the bottom, the tissue and cells were digested with, %: trypsin 0.15 and EDTA 0.02 at 37°C in a shaking bath. The dispersed cells were filtrated through a 150-μm mesh and then seeded in 6-well culture plates. To ensure the quality of the cell culture, the cultured cells were identified by cytokeratin 18 because the mammary epithelial cells were immunostained positively for the cytokeratin.

Treatment of cultured cells with amino acids

The amino acids were diluted with medium. At the beginning of culture, mammary epithelial cells were treated with different doses of Met (0-100 μg/ml) in medium. Using optimal dose of Met, cells were then challenged with different ratio (1:1-4.5:1) of Lys to Met.

RT-PCR assay

Total cellular RNA was extracted by Trizol (In Vitrogen). The RNA purity was determined by optical density (OD260 nm/OD280 nm absorption ratio >1.80). The α_{s1} casein and GAPDH mRNA was amplify by RT-PCR according to the previous report (Wu et al., 2004). PCR products were analysed by electrophoresis in 1.2%

agarose gel. The net intensities of individual bands were measured using Image Master VDS Software (Pharmacia Biotech, Sweden). The ratio of the intensities of α_{s1} casein to GAPDH represented the relative mRNA level of α_{s1} casein. The average level of three repeats was used for statistical analysis.

Statistical analysis

All data were analysed by ANOVA and Duncan's multiple range tests using the SAS 9.0 software. $P<0.05$ was considered as significantly different.

RESULTS

α_{s1} Casein mRNA expression was increased with the increasing level of Met up to 60 μg/ml, and then leveled off (Figure 1). From the fitted curve of optical density of the electrophoretic α_{s1} casein gene expression (y) and Met level (x, μg/ml): $y=-0.0071x^2+1.5139x+14.855$ ($R^2=0.8692$), it was estimated that the highest abundance of α_{s1} casein mRNA occurred at 57 μg/ml of Met ($P<0.05$).

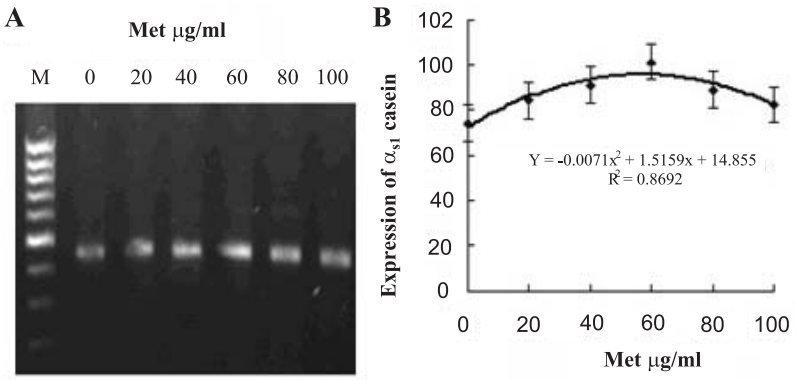

Figure 1. Effects of methionine (Met) levels on α_{s1} casein gene expression in cultured mammary epithelial cells. A - agarose gel electrophoresis. B - means of optical density of the electrophoretic α_{s1} casein gene expression influenced by adding Met

There was an increasing trend in α_{s1} casein mRNA expression with increasing ratio of Lys to Met in mammary epithelial cells (Figure 2). Highest α_{s1} casein mRNA expression was observed at ratio of 3.5:1 ($P<0.05$). However, no obvious changes were found among all groups at the ratio from 3:1 to 4.5:1.

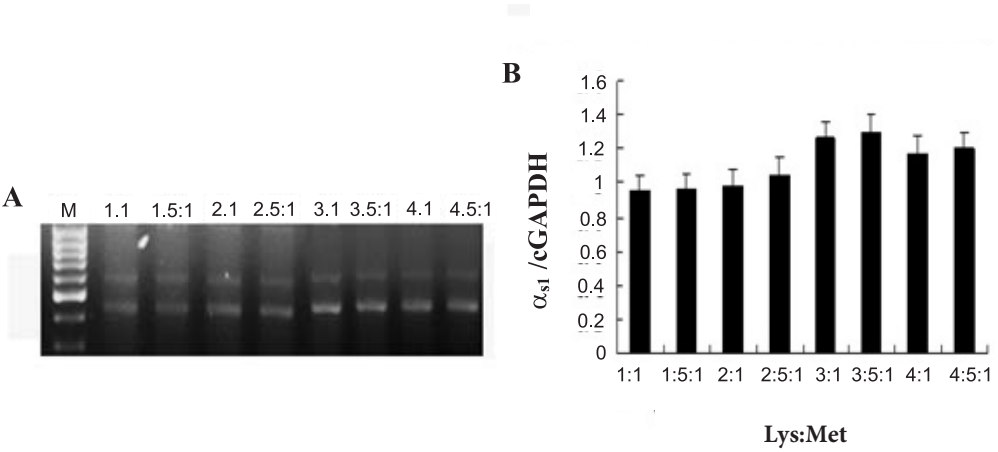

Figure 2. Effects of the ratio of lysine (Lys) to methionine (Met) on α_{s1} casein gene expression in cultured mammary epithelial cells. A - agarose gel electrophoresis. B - means of optical density of the α_{s1} casein gene expression influenced by Lys to Met ratio

DISCUSSION

The lactating mammary gland has a large demand for AA to meet the requirements for milk protein synthesis. Identification of AAs that limit milk protein synthesis has been a focus of ruminant nutrition studies for a long time. These studies have been interpreted collectively as suggesting that Met and Lys are generally limiting milk protein synthesis (Schwab et al., 1992). The most direct evidence of their limitation has been observed by infusing individual or combination of AAs into the abomasum or duodenum and measuring effects on N retention and milk protein production. Responses of lactating dairy cows to improved supplies of Met and Lys in metabolizable protein (MP) include variable increases in milk protein contents and yield (Garthwaite et al., 1998).

The mammary cells can respond to changes in nutritional adequacy of its external and internal AA environments by altering the activities of the AA transport systems. Therefore, this system was sensitive to the exogenous nutriments and could be used as a model for evaluating actions of AA and peptides on protein synthesis *in vitro* (Barnett et al., 2004; Zhao et al., 2004).

In this study, mammary epithelial cells were adopted to evaluate the effects of Met and its ratio to Lys on α_{s1} casein mRNA expression. The α_{s1} casein gene was found to most abundantly express at 57 μg/ml of Met. There was an increasing trend in α_{s1} casein mRNA expression when the ratio of Lys to Met increased from

1:1 to 4.5:1. The α_{s1} casein gene expression showed the highest level at the ratio of 3.5:1 of Lys to Met. These results indicated that appropriate quantities and ratio of Met and Lys significantly increase the synthesis of milk protein. These results were in agreement with the viewpoints of Wu et al. (1999) and the NRC (2001) recommendations for the maximum use of Lys and Met for milk production. Imbalances created by excesses or deficiencies of dietary AA may limit milk protein synthesis and reduce lactation performance. Therefore, adequate quantities and ratio of AA are required to promote milk protein synthesis and its yield.

In summary, the mammary epithelial cell may be a suitable *in vitro* model to study AA metabolism. The α_{s1} casein mRNA expression was most abundant at the Met level of 57 μg/ml and showed the highest level in the ratio of 3.5:1 of Lys to Met.

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