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Effects of methionine-containing dipeptides on α_{s1}casein expression in bovine mammary epithelial cells*

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ABSTRACT

Effects of methionine-containing dipetides on αs1 casein gene expression were investigated in cultured bovine mammary epithelial cells. α_{s1}Casein gene expression was higher in the culture added with methionylmethionine than in that with methionine. Addition of methionyllysine enhanced αs1 casein gene expression, compared with methionine plus lysine. These results indicated that mammary epithelial cells can utilize methionine-containing dipeptides for milk protein synthesis with a higher efficiency than the respective amino acid.

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

INTRODUCTION

Mammary cells are able to utilize amino acids bound to peptides for tissue or milk protein synthesis (Backwell et al., 1996). Supplementation of rumen protected methionine (Met) can improve the efficiency of milk protein synthesis (Nichols et al., 1998; Xu et al., 1998). However it is still uncertain if the Met-containing dipeptides affect the milk protein synthesis. This study was conducted to investigate the effects of methionylmethionine (Met-Met) and methionyllysine (Met-Lys) on α_{s1}casein gene expression in cultured bovine mammary epithelial cells.

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MATERIAL AND METHODS

Reagents

DMEM/F12 was purchased from Gibco BRL Life Technologies. Prolactin, insulin, transferrin, trypsin were supplied by Sigma; foetal calf serum, and hydrocortisone were obtained from Sangon (Shanghai). Reagents for reverse transcriptase (RT)-PCR were obtained from Invitrogen. Methionine and lysine were obtained from AMRESCO; Met-Met and Met-Lys (purity>99.8%) were supplied by Zhongtai Biochemistry (Hangzhou). All other reagents were of the highest purity commercially available.

Preparation and culture of 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 then plated in collagen-treated 6-well culture plates and incubated at 37°C under 95% air and 5% CO_2. The procedures of dispersion and culture of mammary epithelial cells were carried out. Briefly, when cells covered 80% of the plates, the tissues were digested with 0.15% trypsin and 0.02% EDTA. The dispersed cells were filtrated through a 105 μm mesh and then seeded at a density of 5×10^4 cells/ml in DMEM/F12 medium. The basal medium was replenished with 2 μg/ml hydrocortisone, 5 μg/ml transferrin, 10 μg/ml insulin, 1% glutamine, 1% penicillin and streptomycin, and 10% foetal calf serum.

Treatments

The optimal concentration of adding methionine was at 60 μg/ml for the expression of α_{s1} casein gene in cultured mammary epithelial cells (Yang et al., 2007). Lysine was also usually considered as a limiting amino acid. The proportion of lysine in amino acids of α_{s1} casein is triple of methionine (GENE BANK, M38641). In order to investigate effects of methionine-containing dipeptides on α_{s1} casein gene expression, the basal culture mediums were added with 60 μg/ml Met-Met in comparison to 60 μg/ml methionine, and with 120 μg/ml Met-Lys in comparison to 60 μg/ml methionine plus 60 μg/ml lysine, respectively.

Methods

Total cellular RNA was extracted by Trizol (Invitrogen). The RNA purity was determined by optical density (OD260 nm/OD280 nm absorption ratio >1.80). The RT-PCR was used to amplify the mRNA of α_{s1} casein and GAPDH in the

system, and the procedures of RT-PCR were according to the previous reports (Wu et al., 2004). PCR products were analysed by electrophoresis on 1.2% agarose gel. The net intensities of individual bands were measured using Image Master VDS Software (Pharmacia Biotech, Sweden). The average level of three repeats was used for statistical analysis.

Statistical analysis

Statistical analyses were undertaken using the GLM procedure of SAS (2000). $P<0.01$ was considered as significantly different.

RESULTS

This study adopted an *in vitro* model of mammary epithelial cells to evaluate the effects of methionine-containing dipeptides on α_{s1} casein expression. Addition

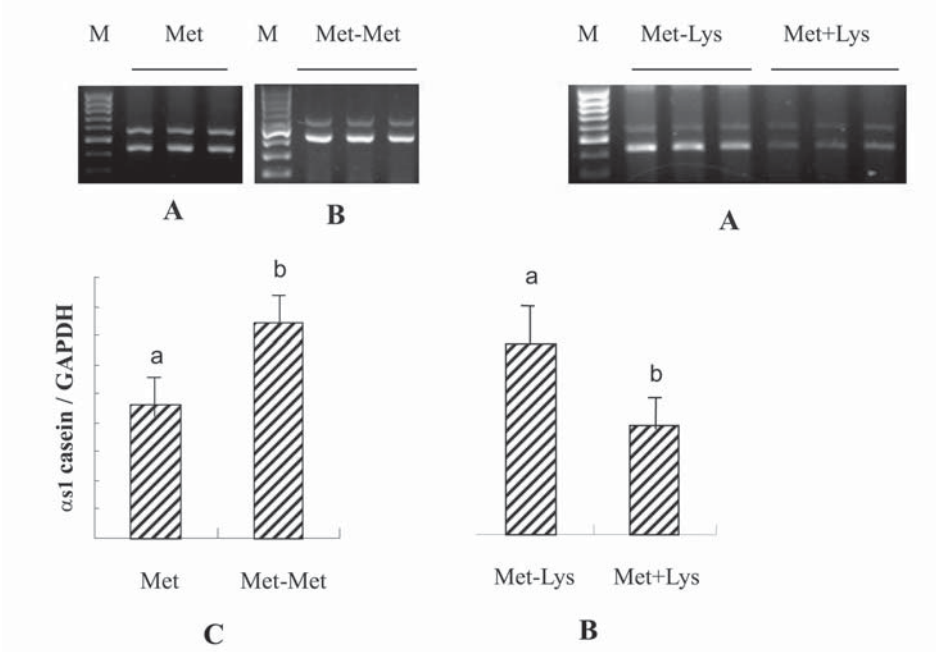

Figure 1. Comparison of the effect of methionine (Met) and methionylmethionine (Met-Met) on α_{s1} casein gene expression in bovine mammary epithelial cells. A and B - agarose gel electrophoresis. C - means of optical density ratio of electrophoretic α_{s1} casein to GAPDH. ^{a,b} $P<0.01$

Figure 2. Comparison of the effect of methionine (Met) plus lysine (Lys) and methionyllysine (Met-Lys) on α_{s1} casein gene expression in bovine mammary epithelial cells. A - agarose gel electrophoresis. B - means of optical density ratio of the electrophoretic α_{s1} casein to GAPDH. ^{a,b} $P<0.01$

of 60 μg/ml Met-Met promoted the α_{s1} casein expression in cultured mammary epithelial cells as compared with 60 μg/ml methionine-supplemented group (Figure 1; $P<0.01$). Similarly, α_{s1} casein expression on 120 μg/ml of Met-Lys was more abundant than that on 60 μg/ml of methionine plus lysine (Figure 2; $P<0.01$). It is suggested that mammary epithelial cells can utilize dipeptides to synthesis milk protein. Furthermore, the efficiency of utilization of methionine-containing dipeptides is higher than free amino acids.

DISCUSSION

In the current study, addition of Met-containing dipeptides as source of methionine and lysine promoted the α_{s1} casein expression in cultured mammary epithelial cells as compared with respective free amino acids. The results is consistent with the findings that histidine-containing dipeptide enhanced milk protein production compared with free histidine (Backwell et al., 1996). The mechanism for utilization of dipeptides is complicated and still unclear. It has been reported previously that cultured mammary epithelial cell can utilize peptides containing methionine as a substitute for free methionine supplied for mammary tissue and secretory protein synthesis (Pan et al., 1996). In the experiment with rats *in vivo* by Shennan et al. (1998), it is observed that dipeptides were hydrolysed extracellularly followed by uptake of the constituent amino acids. However, it is hard to explain the significantly increased efficiency of gene expression when the dipeptide containing methionine was used as supplement of methionine, as the expression of α_{s1} casein would not increase when the concentration of free methionine was higher than 60 μg/ml in cultured mammary epithelial cell (Yang et al., 2007).

The methionine transporters may be saturated at 60 μg/ml, but intact dipeptides may be absorbed through differential dipeptide transporter located at the membrane of bovine mammary epithelial cells (Groneberg et al., 2002). The peptide transporter has been found in mammary gland of rats and rabbits. Although the differences between species and methods should be taken into consideration, there should exist different dipeptide transporter on the membrane of bovine mammary epithelial cells. α_{s1} casein contained Met-Lys sequence (GENE BANK, M38641), thus the dipeptides absorbed by epithelial cells may be directly used to synthesize casein.

CONCLUSIONS

Methionine-containing dipeptides can be utilized by mammary epithelial cells, and the efficiency of utilization was higher than that of equivalent free amino acids. The increased efficiency of casein α_{s1} may have resulted from both enhanced

α_{s1} casein gene expression and direct use of these dipeptides by bovine mammary epithelial cells.

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