

# Impact of free lysine on reactive lysine measurement by guanidination

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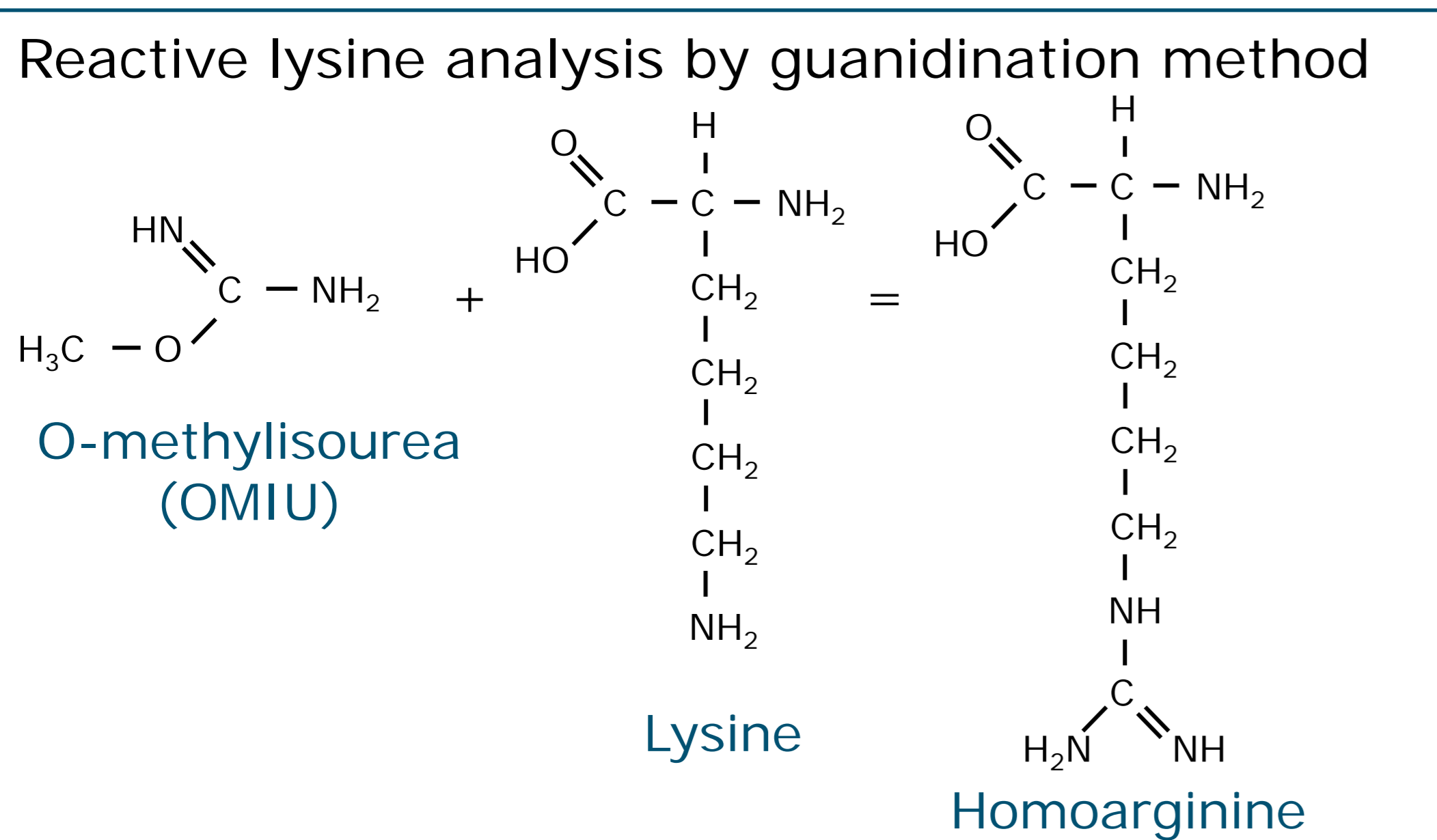
## Objective

- To investigate the specificity of O-methylisourea (OMIU) for the  $\epsilon$ -amino group of crystalline L-lysine

## Introduction

Analyzed lysine (acid hydrolysis) = reactive lysine + reverted lysine

- Reactive lysine = lysine with a free  $\epsilon$ -amino group
  - Not reacted to sugars or amino acids
- Reverted lysine = lysine from early Maillard reaction products
  - Unavailable for protein synthesis in animals<sup>1</sup>



Specificity of OMIU was questioned

- OMIU might bind to  $\alpha$ -amino groups of several amino acids<sup>2,3</sup>
- Underestimation of reactive lysine in diets containing L-lysine HCl<sup>4</sup>

## Methods & Results

### Step 1. Crystalline L-lysine

Methods – OMIU incubation

- OMIU pH 10.6
  - OMIU to lysine ratio of 1000:1
  - 7 day reaction time
- High-performance liquid chromatography analysis

Results – Recovery of ...

- Unreacted lysine (free  $\alpha$ - and  $\epsilon$ -amino group) 2%
- Homoarginine (free  $\alpha$ -amino group) 2%
- Non-recovered lysine 96%
  - bound  $\alpha$ - and  $\epsilon$ -amino group?

### Step 3. Can the reaction be modified to get 100% homoarginine recovery?

Methods – Parameters tested

- OMIU to L-lysine ratio (1.5, 10, 100 or 1000:1)
- Reaction time (1, 3 or 7 days)
- OMIU pH (8.6 to 11.0 with steps of 0.4)

High-performance liquid chromatography analysis

Results

- None of the tested parameter combinations gave 100% homoarginine recovery and 0% unreacted lysine recovery

## Conclusions

- OMIU binds to the  $\alpha$ -amino group of lysine
- OMIU is not specific for  $\epsilon$ -amino group of lysine
- Altering OMIU pH, OMIU to amino acid ratio and reaction time does not result in absolute specificity

## Recommendations

- Analyze protein-bound lysine using OMIU pH of 10.6, OMIU to lysine ratio of 1000:1 and 3 days reaction time
- Analyze free lysine content separately

### Step 2. LC/MS of L-lysine

Methods – OMIU incubation

- OMIU pH 10.6
- OMIU to lysine ratio of
  - 10:1, 100:1 or 1000:1
- 3 day reaction time

Ultra high-performance liquid chromatography and mass spectrometry analysis

Results – Figure 1. MS spectra of LC peaks at 1.79 min (A) and 2.94 min (B)

