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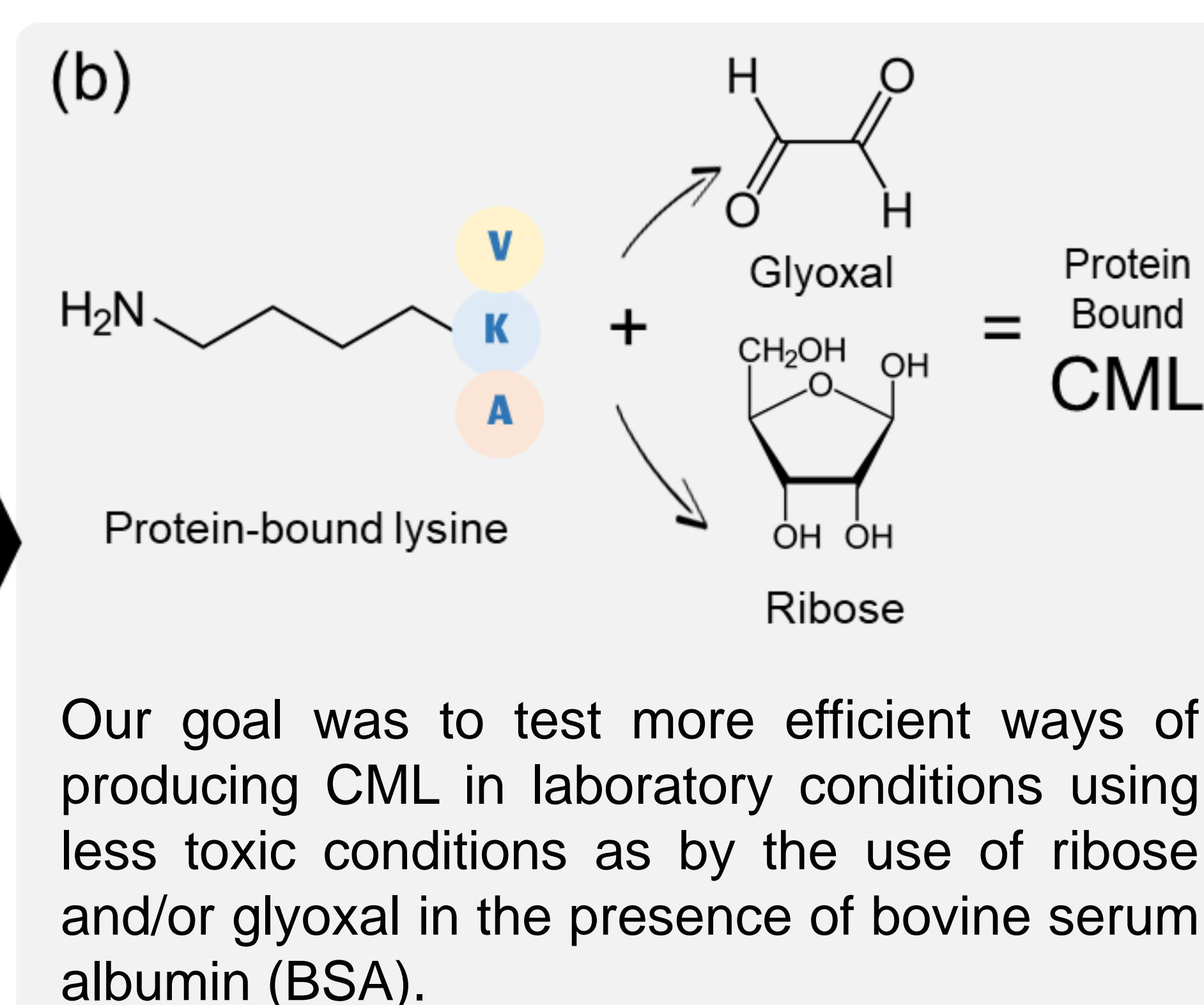
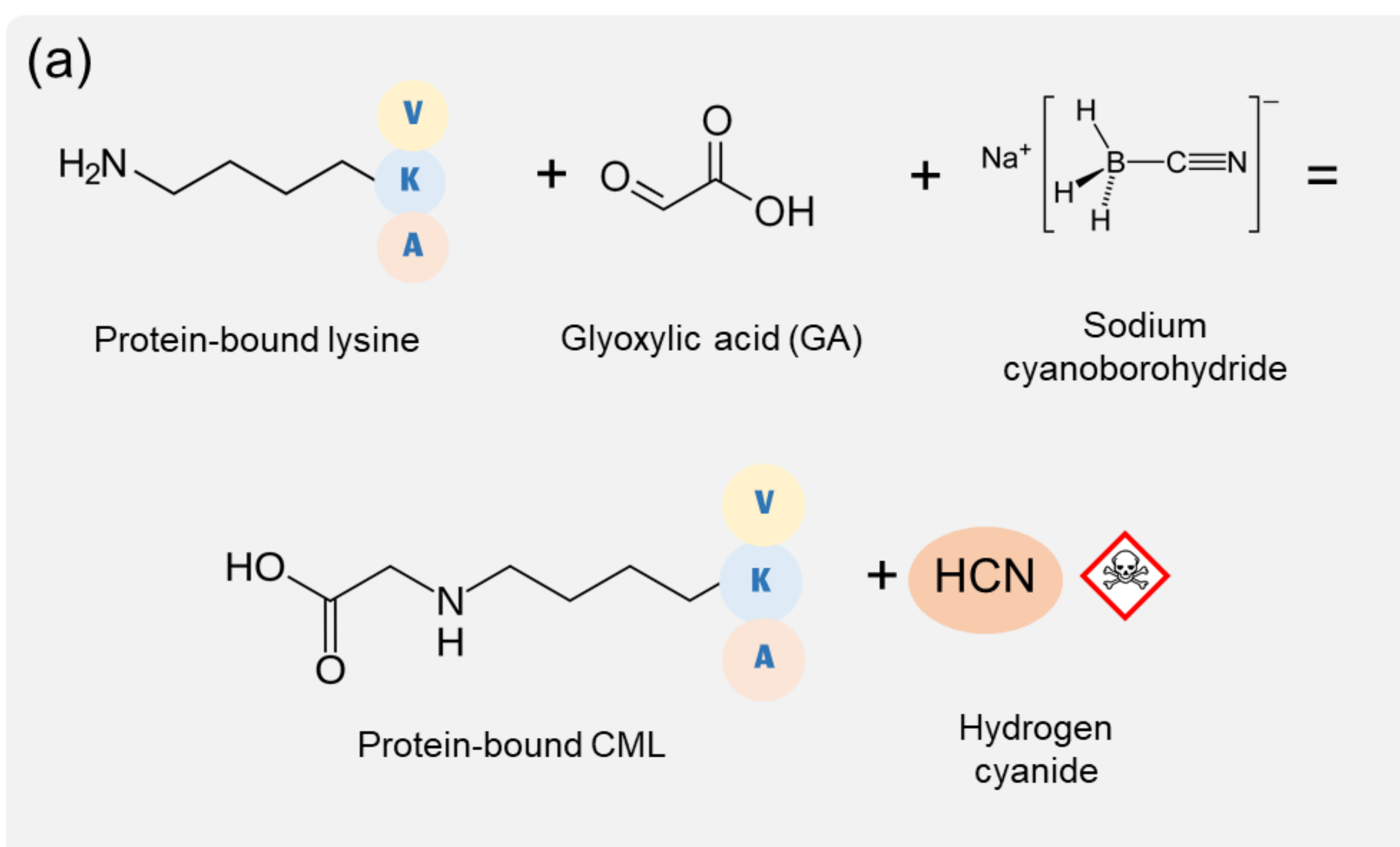
In vitro fortification of N ϵ -carboxymethyl-lysine in bovine serum albumin model systems

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Introduction

The heat treatment of foods results in the production of a heterogeneous group of glycated molecules related to the flavor and aroma of foods, but associated with potential negative health effects. Carboxymethyl-lysine (CML), result of the glycation of lysine residues, is a major dietary advanced glycation end-products (AGE) for the investigation of the effects of AGE consumption on human health (1).



Methods

Traditional reducing agents (2)	Alternatives (3)
> NaBH ₄	> Ribose
> NaNH ₃ CN	> Glyoxal
> NaBH(OAc) ₃	

Western blot

LC-MS/MS (Acid hydrolysis)

Proteomics (Enz. hydrolysis)

Reaction conditions: phosphate buffer (0.2M, pH 8.0) - 37°C for different periods.

Figure 1. Traditional (a) and alternative (b) methods on the production of protein-bound CML.

Results

Degree of lysine modification into CML(%) in BSA using traditional methods:

Reagent	CML formation (% of lysine)
NaNH ₃ CN	37.0
NaBH ₄	3.5
NaBH(OAc)	1.6

Ribose with or without glyoxal induced a loss of 35 to 57% of lysine, but low CML formation (1-2%).

The CML fortification of BSA with increasing concentrations of glyoxal alone formed CML in proportion to the glyoxal concentration: 0.5, 1.0, 4.5, 9.8 and 18% of lysine with 20, 40, 100, 200 and 300 mM glyoxal, respectively (Fig. 2a).

Further characterization:

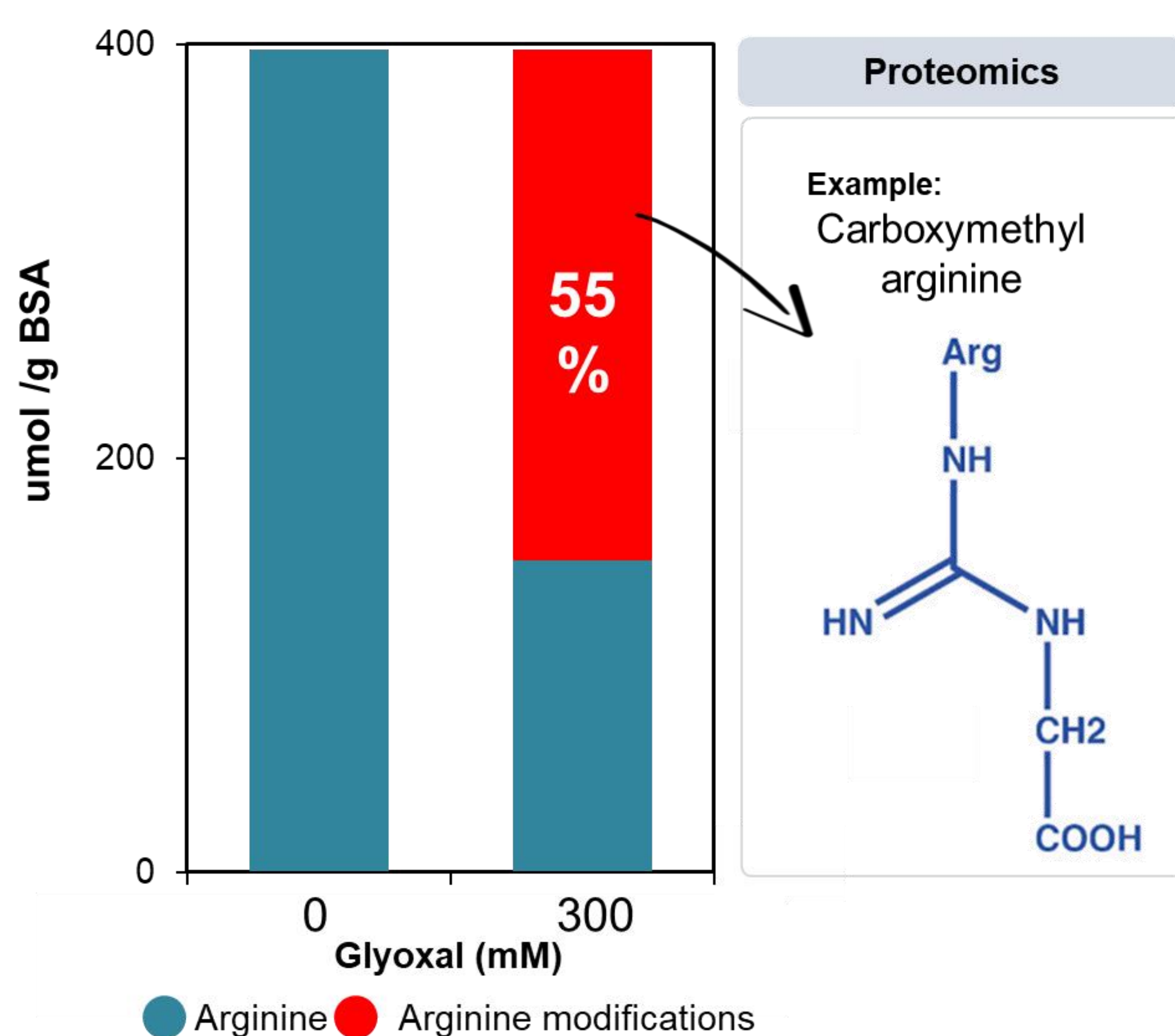


Figure 3. Glycation between BSA and glyoxal. Assessment of arginine modifications by LC-MS/MS.

Proteomic analysis identified arginine modifications as carboxymethyl-arginine in this preparation (Fig. 3).

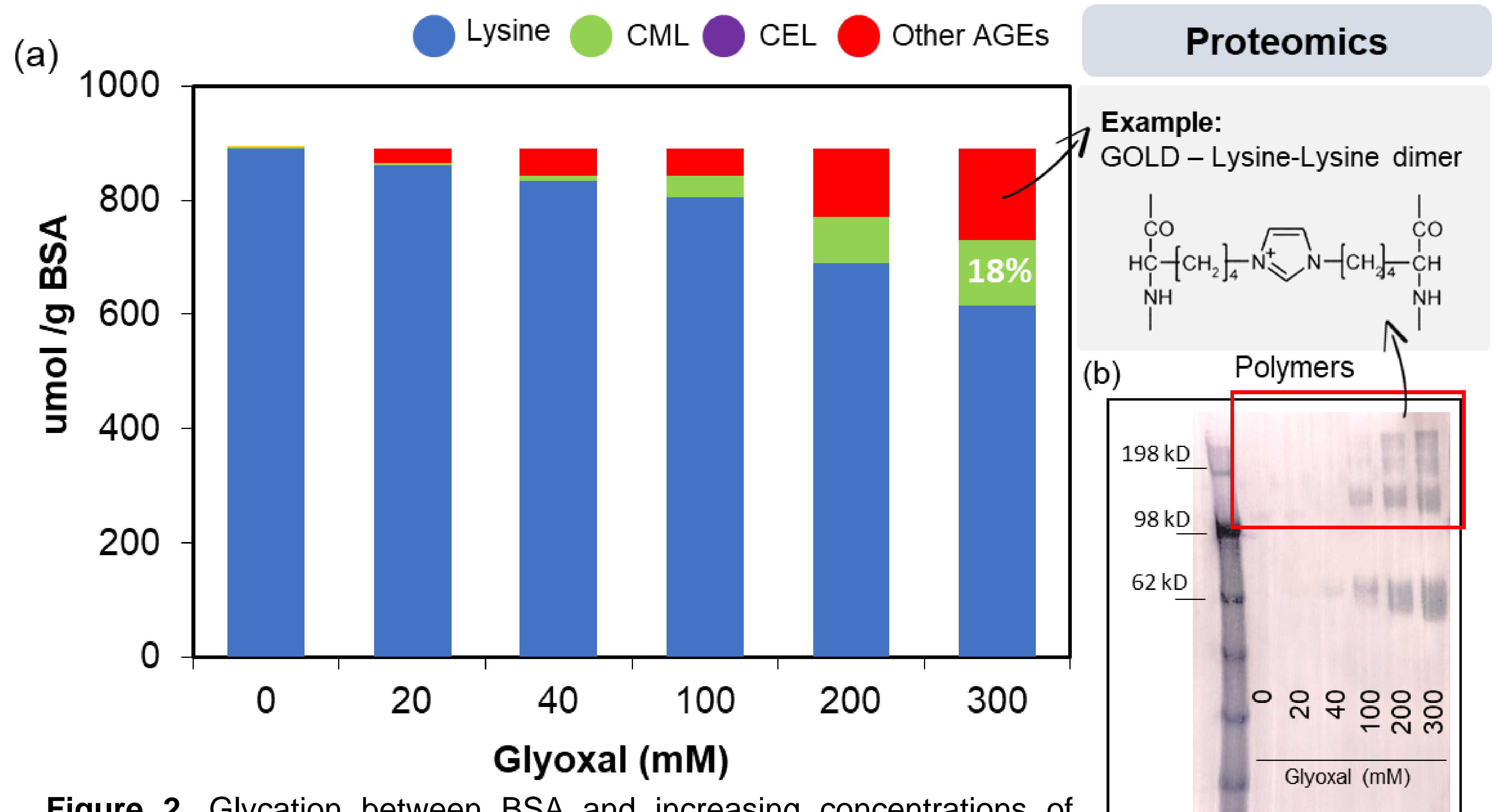


Figure 2. Glycation between BSA and increasing concentrations of glyoxal (0 to 300 mM). (a) LC-MS/MS quantification of CML, carboxyethyl-lysine (CEL), lysine, and estimation of other AGEs. (b) CML detection on a western blot. Antibody Cell Signaling 4764S (1/5000).

Conclusion

Alternative:

BSA + 300mM glyoxal alone is a suitable alternative to GA with NaBH₃CN

Pros: > No HCN production

> Satisfactory lysine modification into CML (18%) compared to other methods

Perspectives:

- > Finding a model protein selectively fortified only with CML remains to be discovered;
- > Characterize the crosslinks between proteins.

References

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