

# Copper(II)-Complexation by Non Enzymatically Glycated Peptides

S. T. SEIFERT<sup>1,2\*</sup>, R. KRAUSE<sup>1</sup>, K. GLOE<sup>2</sup> and T. HENLE<sup>1</sup>

<sup>1</sup>Institute of Food Chemistry and <sup>2</sup>Institute of Inorganic Chemistry,  
Technical University Dresden, Germany,

\*E-mail: steffen.seifert@chemie.tu-dresden.de

**Abstract:** The purpose of our work was to examine the metal binding abilities of selected peptide bound Maillard reaction products (MRPs). The *N*<sup>α</sup>-hippuryl-protected MRPs *N*<sup>ε</sup>-fructoselysine and *N*<sup>ε</sup>-carboxymethyllysine were synthesised and measurement of stability constants for complexes formed with the physiologically important metal ions copper(II) and zinc(II) was carried out in aqueous solution (T = 298.1 K; I = 0.1M KNO<sub>3</sub>) using pH-potentiometry. The stability constants of *N*<sup>ε</sup>-fructoselysine and *N*<sup>ε</sup>-carboxymethyllysine with Cu(II) proved that new coordination centres are formed by the nonenzymatic glycation of proteins. With zinc(II) no complexation was observed. Physiological consequences are discussed, but further studies are necessary in order to clarify the effects of this phenomenon.

**Keywords:** *N*<sup>α</sup>-hippuryl-*N*<sup>ε</sup>-fructoselysine; *N*<sup>α</sup>-hippuryl-*N*<sup>ε</sup>-carboxymethyllysine; pH-potentiometry; stability constants; Maillard reaction products

## INTRODUCTION

The reaction between reducing sugars and amino compounds, also referred to as Maillard reaction, is an important reaction for food processing and storage and therefore has been an important research area of food chemists for decades. Non enzymatic glycated amino acids and proteins could be found in several kinds of stored or heated food. During food consumption, this Maillard reaction products (MRPs) can be resorbed to a certain extent [1]. Furthermore, MRPs also are formed via glycation processes *in vivo* [2]. Formation of MRPs may have both positive and negative consequences for the human organism [3, 4]. Up to now, very little is known about functional changes of glycated proteins. In this context, it has been assumed that MRPs are able to form complexes with physiologically relevant metal ions. In feeding studies with rats it was shown that MRPs in food decrease the bioavailability of metal ions [5–7]. A clear influence of MRPs on mineral metabolism in humans was shown by STEGINK *et al.* [8]. In comparison with orally administered heat sterilised glucose-amino acid solution, the same solution intravenously ad-

ministered enhances the urinal excretion of zinc, copper and iron two- to fivefold. Complexation of metal ions by MRPs may be responsible for such effects, resulting in altered bioavailability of metal ions due to an influence on absorption, retention, excretion and enzymatic reactions [5, 8].

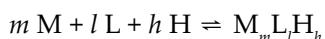
Therefore, the aim of our study was to examine the complex formation of selected peptide bound MRPs of lysine with biologically relevant metal ions. In this paper the stability constants of the quantitative important MRPs *N*<sup>ε</sup>-fructoselysine and *N*<sup>ε</sup>-carboxymethyllysine with the metal ions zinc(II) and copper(II) were determined using pH-potentiometry. Both MRPs were synthesised as the *N*<sup>α</sup>-hippuryllysine (HipLys) derivatives in order to block the known coordination function of the α-amino group and to model peptide bound derivatives concomitantly.

## EXPERIMENTAL

**Syntheses of the MRPs.** The synthesis and isolation of *N*<sup>α</sup>-hippuryl-*N*<sup>ε</sup>-fructoselysine (HipFruLys) and *N*<sup>α</sup>-hippuryl-*N*<sup>ε</sup>-carboxymethyllysine (HipCML) was performed according to literature [9].

**pH-potentiometric measurements.** The potentiometric titration measurements were carried out at  $25 \pm 0.1^\circ\text{C}$  and constant ionic strength ( $I = 0.15 \text{ mol/l}$ ;  $\text{KNO}_3$ ) under a  $\text{CO}_2$ -free moist nitrogen atmosphere. Apparatus, calibration procedure and the carrying out of the test are described in [9].

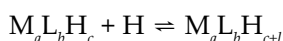
The overall formation constants as well as stepwise protonation and stability constants are reported. Overall formation constants are given as  $\beta_{mlh}$  for the reaction



$$\text{with } \beta_{mlh} = \frac{[\text{M}_m \text{L}_l \text{H}_h]}{[\text{M}]^m \cdot [\text{L}]^l \cdot [\text{H}]^h}$$

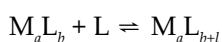
whereas M stands for the metal ion, L the deprotonated ligand and H the proton.

The stepwise protonation constants and stepwise stability constants describe the reaction



$$\text{with } k_{c+1} = \frac{[\text{M}_a \text{L}_b \text{H}_{c+1}]}{[\text{M}_a \text{L}_b \text{H}_c] \cdot [\text{H}]}$$

and



$$\text{with } k_{c+1} = \frac{[\text{M}_a \text{L}_{b+1}]}{[\text{M}_a \text{L}_b] \cdot [\text{L}]}$$

Negative constants for H were used to describe complexes with hydroxides. The hydrolysis constant of a metal ion ( $\beta_{1x0}$  with  $x \text{ OH}^-$  as ligands) for example results from:  $\beta_{1x0} = \beta_{10-x} \cdot x \cdot K_w$ .

## RESULTS AND DISCUSSION

Due to the desalting step of the synthesis, certain amounts of acetic acid were associated with MRPs which has to be taken into account. The acidity constant  $\text{p}K_a = 4.55$  (0.01) corresponds with literature data ( $\text{Log}_{10} K_1 = 4.56 \pm 0.03$ ,  $I = 0.1 \text{ M}$ ,  $T = 298 \text{ K}$ , [10]). Significant complexes with Cu(II) and Zn(II) could not be found with acetic acid, because just very weak complexes are formed [10]. On the basis of pH-potentiometry experiments, the overall formation constants for HipFruLys and HipCML with Zn(II) and Cu(II) were quantified (Table 1).

The protonation of the free ligands is similar, whereas the individual constants are clearly influenced by the presence of the adjacent functional

Table 1. Overall formation constants for protonation and complexation of HipFruLys, HipCML and HipLys with Cu(II) at 298.1 K and  $I = 0.15 \text{ M}$  ( $\text{KNO}_3$ )

$\text{Log}_{10} \beta_{mlh}$	HipFruLys	HipCML	HipLys
<b>Protonation constants</b>			
$\text{Log}_{10} \beta_{011}$	8.81 (0.02)	9.91 (0.03)	10.62 (0.01)
$\text{Log}_{10} \beta_{012}$	12.00 (0.02)	13.66 (0.05)	13.75 (0.01)
$\text{Log}_{10} \beta_{013}$	— —	15.67 (0.05)	— —
<b>Stability constants with Cu(II)</b>			
$\text{Log}_{10} \beta_{110}$	— —	7.34 (0.07)	— —
$\text{Log}_{10} \beta_{120}$	— —	13.68 (0.04)	— —
$\text{Log}_{10} \beta_{11-1}$	-0.89 (0.04)	— —	— —
$\text{Log}_{10} \beta_{11-2}$	-7.90 (0.04)	— —	— —
$\text{Log}_{10} \beta_{12-2}$	-3.90 (0.09)	— —	— —

Charges are omitted for clarity; standard deviations in parentheses

groups. These differences are also reflected in the stability constants. HipFruLys forms moderately stable complexes with Cu(II) ( $\text{Log}_{10} K_1 \{[\text{CuL}]/[\text{Cu}]/[\text{L}]\} = 5.82$ ;  $\text{Log}_{10} K_2 \{[\text{CuL}_2]/[\text{CuL}]/[\text{L}]\} = 4.00$ ) whereas HipCML forms some more stable complexes with Cu(II) ( $\text{Log}_{10} K_1 \{[\text{CuL}]/[\text{Cu}]/[\text{L}]\} = 7.34$ ;  $\text{Log}_{10} K_2 \{[\text{CuL}_2]/[\text{CuL}]/[\text{L}]\} = 6.34$ ). No complex formation of Zn(II) with either MRP was observed.

To show that the copper(II) complexation is not a consequence of mechanisms according to the well known Biuret reaction,  $N^\alpha$ -hippuryllysine (HipLys), which was the initial compound for the MRP syntheses, was also analysed (Table 1). HipLys is a model for peptide-bound lysine, HipFruLys and HipCML are the corresponding derivatives of peptide-bound lysine. The stability constants of the glyated products compared with HipLys show that glycation results in the formation of a new Cu(II) coordination centre, whereas Zn(II) is not bound.

This new copper binding centres at peptide bound  $N^\epsilon$ -fructoselysine and  $N^\epsilon$ -carboxymethyllysine form complexes with Cu(II) which are 100 to 1000 times more stable compared to the well known imidazole binding site of peptide bound histidine (references:  $N^\alpha$ -acetylhistidine and  $N^\alpha$ -acetylglycylhistidine; [11, 12]). The Cu(II) species with the present glyated products in dependence of pH is shown in Figures 1 and 2. It can be seen that the MRPs form complexes with Cu(II) within a physiological pH-range.

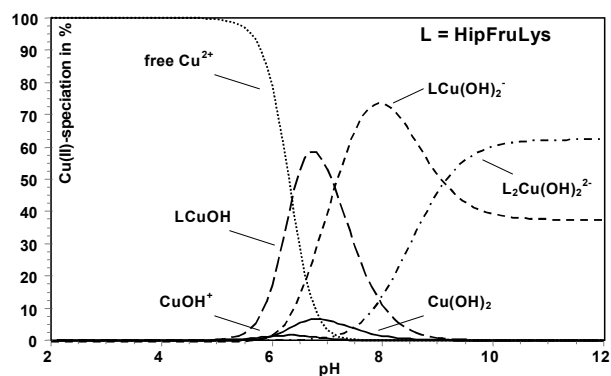


Figure 1. Cu(II) speciation for 1.5mM HipFruLys (metal ion to ligand concentration ratio 1:3)

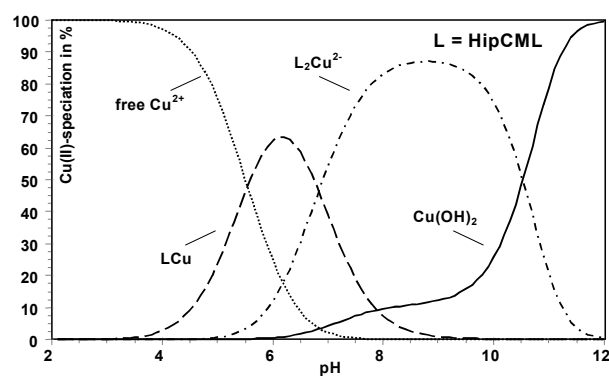


Figure 2. Cu(II) speciation for 1.5mM HipCML (metal ion to ligand concentration ratio 1:3)

In terms of the stability constants, Maillard modification of proteins leads to increased copper binding within the protein chain. As the ascertained stability constants are thermodynamic values, they are in competition with the stability constants of all physiological processes and also in competition with kinetic values. Based on this, an influence on the bioavailability of copper due to complexation by MRPs may be possible, which may influence the plasma metal concentration by the enhanced urinary excretion of MRPs, lead to decreased resorption due to indigestible MRPs or increased resorption due to resorbed MRP-copper complexes. Depending on the stability constants of copper bound in the active center of metalloenzymes, complexation by MRPs might have inhibiting effects. Binding of the redox-active Cu(II) by proteins could also be related to increased oxidative stress, as metal catalysed oxidation was found in some carboxymethyl-rich tissues [13].

## CONCLUSIONS

In summary, these results show that post-translational modification of proteins by carbohydrates leads to the formation of new and effective coordination centres for metal ions within a protein chain. Such complexes may influence the bioavailability, might have inhibiting effects on metalloenzymes and may enhance oxidative stress. Further studies are necessary in order to clarify the consequences of such metal ion binding to MRPs for protein quality and physiological processes.

**Acknowledgement:** We thank the Deutsche Forschungsgemeinschaft (German Research Foundation) for financial support within GK-155.

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