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Methylglyoxal modification hinders amyloid conversion of prion protein

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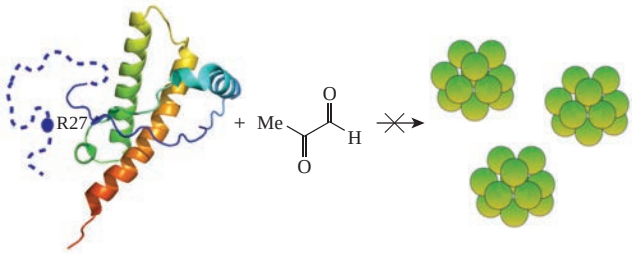
Effect of glycation by methylglyoxal on prion protein (PrP) structure and properties was evaluated. Modification of arginine at 27-position into a hydroimidazolone derivative was confirmed by MALDI-TOF mass spectrometry; circular dichroism spectra and tryptophan fluorescence showed some structural changes, while the hydrodynamic diameter of PrP was not affected by glycation. Glycated PrP formed large amorphous aggregates instead of intermediate oligomers; seeding of glycated PrP by mature fibrils led to a decreased formation of amyloid structures.



Protein glycation occurring under hyperglycemia caused by diabetes mellitus is involved in a development of various pathologies.[1–6] The glycation of proteins might also play an important role during the genesis and development of amyloid-related neurodegenerative disorders, such as prion diseases, Parkinson and Alzheimer's diseases,[7] *etc.* However, with the abundance of information on protein glycation,[8–10] there is a lacuna in the specific molecular mechanisms of this process, especially at the level of individual proteins. The non-enzymatic protein glycation caused by reducing sugars (*e.g.*, glucose, fructose, and galactose) is known to proceed *via* the Schiff bases formation. The next steps are the Amadori rearrangement (which produces 1-amino-deoxy ketose) and a cascade of reactions finally forming the so-called 'advanced glycation end products' (AGEs). After oxidative degradation of Amadori products, dicarbonyl compounds, such as 3-deoxyglucosone, methylglyoxal (MGO), and glyoxal, are formed.[11,12] These highly reactive compounds can react with amino, sulfohydryl and guanidine groups of proteins, causing their modification. This work was aimed at the prion protein (PrP) modification by MGO and the study of concomitant effects, including identification of the affected sites in PrP, the evaluation of physicochemical properties of modified protein, and its ability to form amyloid aggregates of different types.

The glycation of PrP[†] was carried out[‡] using MGO in the 0–2.5 mmol dm^{-3} concentration range. The formation of Amadori products was determined by the reaction with tetrazolium blue;[§] the other experiments were performed using the two selected PrP/MGO concentration ratios [Figure 1(*a*)]. The formation of AGEs after 20-hour modification was confirmed by their intrinsic fluorescence at 335 nm excitation wavelength [Figure 1(*b*)]. The dynamic light scattering measurement of PrP hydrodynamic

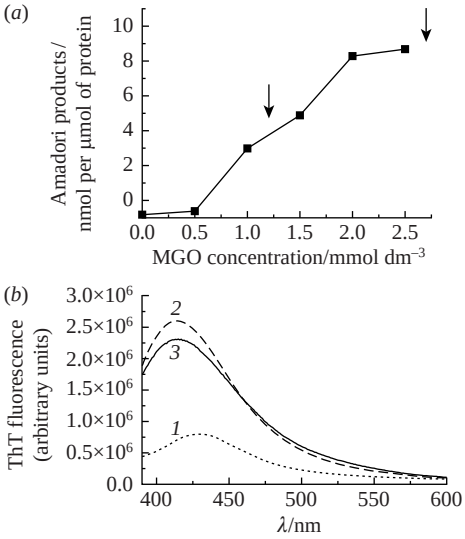


Figure 1 Detection of Amadori products after MGO-modification of PrP by (*a*) NBT assay and (*b*) AGEs fluorescence for (*1*) unmodified, (*2*), (*3*) modified PrP with PrP/MGO molar ratios (*2*) 1 : 31, (*3*) 1 : 75. The arrows highlight the selected PrP/MGO molar ratios (1 : 31 and 1 : 75).

[†] The ovine recombinant prion protein [variant PrP VRQ: V136, R154, Q171; the full-length of its amino acid sequence is 23–234 (pH 7.5); without N-terminal signal and C-terminal peptides; and with one additional Ser residue at the N-terminus] was expressed and purified according to the known procedure.[13]

[‡] PrP was dissolved in sodium acetate buffer (20 mM, pH 4.0) and transferred into potassium phosphate buffer (10 mM, pH 7.4) using a Sephadex G25 column. PrP (40 μM) was incubated with methylglyoxal (1.25 mM and 3 mM, unless precised) at 37 °C for 20 h.

[§] To determine Amadori products content, the tested solution (100 μl) was added to a solution (900 μl) containing nitro blue tetrazolium (0.25 mM) in sodium carbonate (100 mM, pH 10.8), and the sample absorption was measured at 530 nm after 10 min of incubation at 37 °C.

diameter demonstrated that the MGO modified protein retained the hydrodynamic diameter of 6.5 nm, which indicated its monomeric state.

The overall change in the molecular weight of PrP modified with MGO at a small reagent concentration was analyzed using mass spectrometry in order to identify the most prone to modification residues. The peaks with an increased molecular weight (by 54 and 110 Da) were observed [Figure 2(*a*)]. After analysis of fragments from limited trypsinolysis, it was found that arginine residue Arg27 underwent transformation into hydroimidazolone [Figure 2(*b*)]. Moreover, the detected at 6852 and 6906 Da peaks corresponding to the modified and unmodified peptide, respectively, also indicated a possible modification for arginine residue Arg51. Both modified arginines are located in the unstructured N-terminal region of PrP molecule. However, the structured PrP part was changed after modification according to the data for the glycated protein. Thus, a decreased ellipticity was detected after protein glycation in the circular dichroism spectra at 195 and 210–212 nm [Figure 3(*a*)], which denoted the decreased content of alpha-helices and increased content of unstructured areas. Therefore, the glycation with MGO caused changes in the PrP secondary structure by partial unfolding of its alpha helices.

The intrinsic tryptophan fluorescence is known to decrease with increasing number of modifications introduced into the protein [Figure 3(*b*)]. The glycation might cause an alteration of the PrP secondary structure, which accordingly changed the microenvironment around tryptophan residues, finally resulting in the screening of these residues or quenching the Trp fluorescence. In addition, the peak maximum was shifted by 4 nm to a longer wavelength, which might specifically indicate a change in the contribution of Trp residues to the fluorescence spectrum.

We investigated the changes in glycated PrP behavior during the formation of intermediate oligomers[¶] and revealed the formation of huge amorphous aggregates with the diameter about 2000 nm instead of small soluble oligomers with that of 24 nm [Figure 4(*a*)]. Staining with a fluorescent thioflavin T[††] dye (ThT)

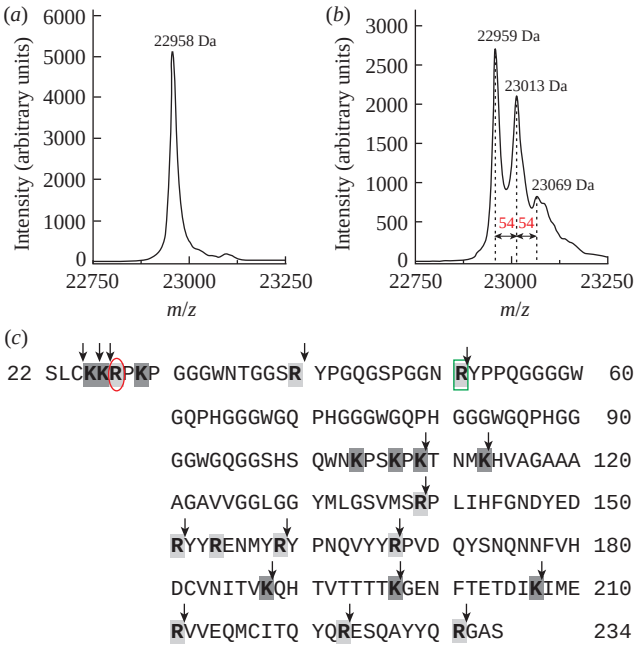


Figure 2 Identification of possible sites for MGO-modified PrP by MALDI-TOF analysis. Molecular weights (*a*) before and (*b*) after modification of PrP (44 μM) by MGO (1 mM) at 1:23 molar ratio at 37 °C for 20 h. (*c*) Sequence analysis of sheep prion (Swiss Prot database, No. P23907, www.expasy.org) using fragments obtained after limited trypsinolysis. All possible cleavage sites for trypsin are indicated by arrows, lysines are marked with dark gray color, and arginines are marked with light gray color. Modification of R27 is encircled and that of R51 is ensquared.

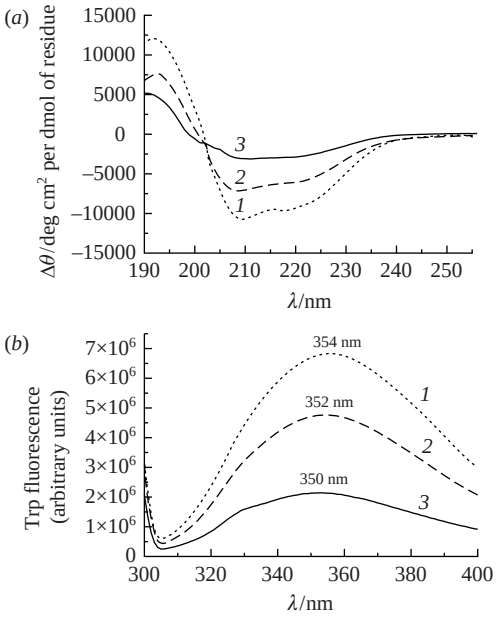


Figure 3 Changes in the physicochemical properties and structure of MGO-modified PrP by (*a*) circular dichroism and (*b*) Trp fluorescence. PrP was diluted in 20 mM sodium acetate buffer for (*1*) control measurement and incubated with MGO for 20 h at (*2*) 1:31 or (*3*) 1:75 molar ratios.

showed an absence of amyloid structures in such aggregates [Figure 4(*b*)].

When mature amyloid fibrils ('seeds') were added to the glycated PrP,[‡‡] the transition efficiency of the normal cellular isoform into an amyloid one was reduced due to a decreased rate of formation of amyloid fibrils, which was measured by the ThT fluorescence [Figure 4(*c*)].

Therefore, our results reveal that the PrP modification by MGO, one of the main *in vivo* glycation adducts, leads primarily to the Arg27 modification and possibly to that for Arg51 as well as the AGEs generation. The glycated PrP did not aggregate under the conditions of a 75-fold MGO molar excess. A slight change observed in the PrP secondary structure was apparently due to the difficulties encountered during the formation of pathological intermediate oligomers and amyloid fibrils in the 'seeding' experiments. It is generally believed that the amyloid protein glycation stimulates pathological processes in the development of amyloid-related neurodegenerative diseases. Such concepts are mostly based on the detection of glycation products in amyloid structures of nerve tissues.[2,18] The PrP oligomeric forms are considered as the most toxic ones. However, this work demonstrates the prevention of their formation after glycation under certain conditions, which indicates an ambiguous role for this type

[¶] To prepare the intermediate oligomers, PrP (40 μM) in MOPS buffer (20 mM, pH 7.5) was incubated at 65 °C for 1 h according to the reported procedure.[14] In case of formation of glycated PrP large amorphous aggregates, they were separated by centrifugation for 5 min at 9000*g*. The pellet and supernatant were analyzed separately.

[††] According to ref. 15, the freshly prepared aqueous thioflavin T (ThT) solution was added to PrP (20 μM) in MOPS (20 μM, pH 7.5) in a molar ratio of 10:1. ThT was incubated with protein samples for 15 min. The ThT fluorescence was measured in 96-well plates at 430 nm (excitation) and recorded close to its maximum at 485 nm (PerkinElmer 2030 Multilabel Reader Victor X5); or the full spectra were recorded on a FluoroMax-3 spectrofluorimeter (Horiba Jobin Yvon, Longjumeau) in a 2 ml cuvette.

[‡‡] Seeding was induced in the solution of PrP (40 μM) in MOPS buffer (20 mM, pH 7.5) by addition of fibrils suspension at the molar ratio of 1:50, with or without inhibitors, and incubated under intensive shaking for 4–7 days.[16] The prion fibrils were obtained using PrP (87 μM) in acetate buffer (100 mM, pH 4.0), containing sodium azide (0.03%) with guanidine hydrochloride (1 M) at 37 °C under shaking and stirring during 2 days.[17]

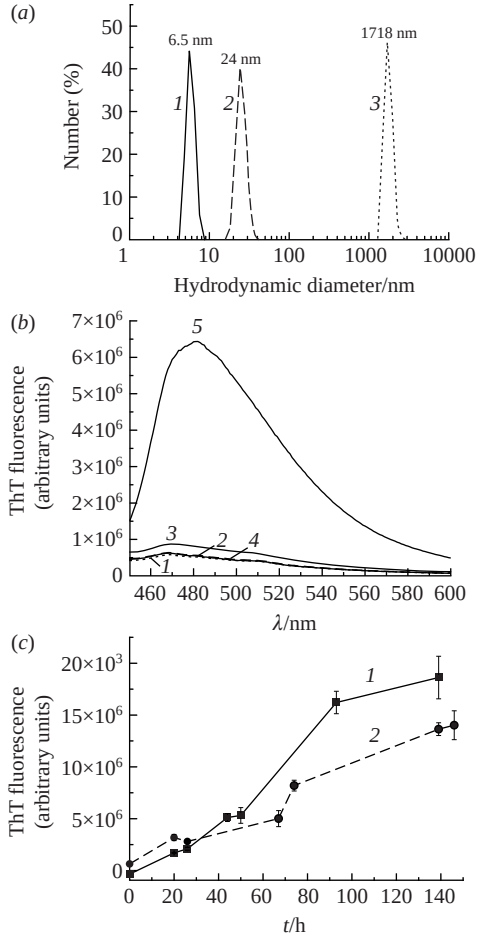


Figure 4 (*a*), (*b*) Impact of MGO-modification on the oligomer formation and (*c*) seeding of PrP. (*a*) Hydrodynamic diameter of (*1*) non-modified PrP, (*2*) PrP oligomers, and (*3*) supernatant after oligomerization in the presence of MGO at 1 : 75 molar ratio. (*b*) ThT fluorescence of (*1*) non-modified PrP, (*2*) PrP oligomers, (*3*) supernatant, (*4*) resuspended pellet after PrP oligomerization of MGO-modified PrP at 1 : 75 molar ratio, and (*5*) amyloid fibrils of non-modified PrP. (*c*) Seeding by mature PrP fibrils of (*1*) non-modified PrP and (*2*) MGO-modified PrP at 1 : 75 molar ratio.

of posttranslational modification in the development of neurodegenerative pathologies.

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