

Received: 2004.07.28
Accepted: 2004.10.22
Published: 2005.02.15

Advanced glycation end-products prepared in solution under high pressure contain epitopes distinct from those formed in the dry reaction at high temperature

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

Magdalena Staniszewska^{1ABDF}, Sławomir Jarosz^{2B}, Marek Jon^{3B}
and Andrzej Gamian^{1ADEG}

¹ Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

² Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland

³ Institute of Chemistry, Wrocław University, Wrocław, Poland

Source of support: grant no. 6 P04A 040 19 of the State Committee for Scientific Research (KBN, Poland).

Summary

Introduction:

Advanced glycation end-products play an important role in diseases related to diabetes and aging processes. Model compounds are synthesized in order to prepare the diagnostic and experimental tools for studying the mechanisms of pathogenesis. The objective of the present study was to accelerate glycation and upgrade its efficiency under high-pressure conditions.

Materials and Methods:

Aqueous solutions of proteins were kept with carbohydrates under a pressure of up to 850 MPa for several hours. Then the high-pressure glycation (HPG) products were fractionated on a Sephadex G-200 column and characterized with SDS-PAGE and MALDI-TOF mass spectrometry.

Results:

The low-molecular-mass fraction of glycated proteins was separated from the two fractions containing high- and intermediate-molecular-mass cross-linked products of glycation. The products were then compared with those obtained with the high-temperature glycation (HTG) procedure carried out in dry conditions with a lyophilized mixture of substrates. The fractionated products were used to prepare rabbit sera.

Conclusions:

The immunoblotting experiments showed that the epitopes on the cross-linked glycation products formed in solution under high pressure differed from those originating in dry conditions at high temperature. Sera against the HPG products were specific to homologous material and did not interact with the fractions obtained by HTG. The antibodies against HTG products recognized HTG but not HPG products.

Key words:

glycation • Maillard reaction • high-pressure reaction • high-temperature reaction • antibodies anti-AGE • epitope

Abbreviations:

AGEs – advanced glycation end-products, **BSA** – bovine serum albumin, **GLC-MS** – gas-liquid chromatography-mass spectrometry, **HPG** – high-pressure glycation, **HTG** – high-temperature glycation, **MALDI-TOF** – matrix-assisted laser-desorption ionization-time of flight, **lac** – lactose, **Mb** – myoglobin, **rib** – ribose, **PBS** – phosphate-buffered saline.

Full-text PDF:

http://www.aite-online/pdf/vol_53/no_1/6821.pdf

Author's address:

Prof. Andrzej Gamian, Ph.D., Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland, tel: +48 71 337 11 72, fax: +48 71 337 13 82, e-mail: gamian@iitd.pan.wroc.pl

INTRODUCTION

Advanced glycation end-products (AGEs), which form during the non-enzymatic glycation (Maillard reaction²⁸) of proteins in organisms are involved in diabetic complications and aging processes^{5, 25, 26}. Glycation begins with the reaction of the aldehyde group of sugar with a free amino group of protein⁷, and a reversible Schiff base occurs which slowly undergoes a rearrangement to the Amadori adduct. Additional dehydration, oxidation, and rearrangements lead to the structurally heterogeneous products, irreversibly bound to proteins. The AGEs accumulated in living tissues on several long-lived proteins, such as collagen²⁴, hemoglobin⁶, and serum proteins^{4, 11, 22}, play an important role in protein turnover, tissue remodeling, and the pathologies of different diseases.

The group of glycation products consists of many different structures. They induce many cellular responses by binding to a specific receptor for the AGEs¹³. Only a few of the possible existing structures, for example pentosidine, carboxymethyllysine, carboxymethylarginine, pyrraline, and several other species, have been defined so far²⁸. They are usually synthesized *in vitro* by long exposure of proteins to sugars or sugar fragmentation products²³ at 37°C or high temperature³. Another way to enhance the reaction can be the application of high-pressure or ultrasonic waves, which affect the internal interactions exclusively by changing the distances (volumes) of the components at constant temperature² and by which some unique products with new properties could be obtained^{12, 18}.

The aim of our study was the application of high-pressure in the synthesis of model AGEs and their characterization by serological analysis compared with those of the known high-temperature synthesis. The mixture of the products was fractionated into a group of low-molecular-mass products and two groups of a cross-linked material, which are considered to be the intermediate- and high-molecular-mass AGEs. With the reported procedure of preparation it was possible to obtain the AGEs within a relatively short time (a few hours) and to carry out the reaction at lower temperature. The glycated products appeared to be immunogenic in rabbits but, interestingly, the antibodies against high-pressure glucation (HPG) products recognized only high-pressure products, being immunochemically distinct from the products formed in the dry reaction at high temperature.

MATERIALS AND METHODS

Glycation reactions and fractionation of the products

Monomeric bovine serum albumin (BSA) was prepared from Cohn fraction V suitable for RIA (Sigma, A7888, Poland) by gel filtration on a 1.6×100 cm Sephadex G-200 column (Pharmacia, Sweden) equilibrated with phosphate-buffered saline (PBS) containing 0.02% (w/v) NaN₃, pH 7.5. The product analyzed by SDS-PAGE was separated from the high-molecular-mass material and was found to contain <0.25% of the carbohydrate material, as shown in sugar analysis by gas liquid chromatography-mass spectrometry (GLC-MS). Myoglobin from equine skeletal muscle (Sigma, M0630) was used without purification.

For the HPG, unless otherwise indicated, an equal amount by weight of sugar (18 mg of lactose, ribose or fructose) was added to a solution of protein (BSA or myoglobin) in water (18 mg/1.5 ml). The plastic Eppendorf tubes with the reaction mixtures were dipped in hexane and compressed to a pressure which ranged 300–860 MPa at 4°C or room temperature for 2–14 h, using an apparatus produced at the Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw. After decompression, the products obtained were separated from the unreacted substrates on a gel filtration column of Sephadex G-200 equilibrated with PBS containing 0.02% (w/v) NaN₃, pH 7.5. The fractionated material was extensively dialyzed against distilled water and lyophilized or kept as water solutions frozen at –20°C. The fractions were analyzed by SDS-PAGE and MALDI-TOF mass spectrometry.

HTG was carried out according to the method of Boratyński and Roy³. Briefly, proteins and sugars were dissolved in water at a ratio of 2:5 (w/w) and 2.5% dimethylformamide was added. The samples in plastic Eppendorf tubes were frozen at –70°C, lyophilized, and then heated at 116–120°C for 30 min. After cooling, the material was dissolved in 1 ml of water, centrifuged (5 min at 14,000 rpm) to remove the insoluble material, and fractionated on a chromatography column as above.

Spontaneous glycation in solution was also performed by incubation of BSA (15 mg) in water (1 ml) containing lactose (15 mg) and 0.02% (w/v) NaN₃ at 37°C for 4 weeks. Following incubation, the sample was dialyzed to remove unbound sugar and kept frozen at –20°C until assayed.

Preparation of antibodies, SDS-PAGE and immunoblotting

Immune sera were obtained after multipoint intradermal 3-fold immunizations of rabbits with the conjugates in PBS, suspended in Freund's complete adjuvant (1:1, v/v)²⁰. Animals were sacrificed on the 7th–9th day after the last injection and the prepared sera were heated at 56°C for 35 min to inactivate the complement.

SDS-PAGE was performed according to the Laemmli method¹⁵ in a 15, 12.5, or 10% polyacrylamide gel, as described elsewhere¹⁰. The conjugate solution (1 mg/ml) in sample buffer 1 (100 mM Tris-HCl pH 6.8, 20 mM EDTA, 8% SDS, 20% glycerol, 0.005% bromophenol blue) or 2 (62.5 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 5% β -mercaptoethanol, 0.005% bromophenol blue) was boiled for 5 min, and 2 μ g conjugate samples were placed onto the gel. After electrophoresis, the gels were stained with Coomassie Brilliant Blue or were transferred to Immobilon P (0.45 μ m; Millipore, USA) as described in reference¹⁴. Briefly, gels after electrophoresis were transferred onto a membrane in 10 mM Tris, 150 mM glycine, 20% (v/v) methanol, pH 8.3, at 100 V for 1 h. The membrane was dried from methanol and incubated at 36°C for 1 h with rabbit antiserum diluted 1:100 with 20 mM Tris-HCl, 50 mM NaCl, 0.05% Tween-20 buffer (TBS-T), pH 7.5, containing 1% (w/v) BSA. After washing 3 times with TBS (20 mM Tris-HCl, 50 mM NaCl, pH 7.5) for 10 s, the blot was incubated with goat antibodies anti-rabbit IgG conjugated with horseradish peroxidase diluted 1:10,000 with TBS-T, at 36°C for 1 h. The blot was washed 3 times with TBS for 10 s, followed by staining with a 4-chloro-1-naphthol substrate solution in the presence of H₂O₂.

MALDI-TOF mass spectrometry

MALDI mass spectra were recorded on a TOF instrument of Kratos PCKompact SEQ type. Spectra were recorded from 22–50 laser shots (nitrogen laser, 337 nm) with an accelerating voltage of 20 kV in a linear mode. As a matrix, 10 mg/ml of 3,5-dimethoxy-4-hydroxy-cyanminic acid was dissolved in an aqueous acetonitrile solution (50%). Then 1 μ l of the matrix was added to 1 μ l of the sample. The sample was placed on a plate and allowed to dry either by itself or in a gentle stream of nitrogen. The spectra were calibrated using a solution of myoglobin (m/z 16,957) or BSA (m/z 66,500) and cytochrome c (m/z 12,358).

Analytical methods for glycation assaying

Total neutral sugar was determined with the phenol-sulfuric acid reagent⁸ and the quantification of free

amino groups was performed with 2,4,6-trinitrobenzenesulfonic acid (TNBS) reagent¹⁶. Sugar compositional analysis was performed as previously described⁹. Oligo- and polysaccharide samples were hydrolyzed with 1 M HCl at 100°C for 4 h, followed by evaporation under a stream of N₂. For GLC-MS analysis, use was made of a Hewlett-Packard 5971A system equipped with an HP-1 glass capillary column (0.2 mm $\times$ 12 m), a temperature program of 8°/min from 150 to 270°C, and 70 eV ionization potential. Fluorescence emission spectra of advanced glycation end-products in samples were recorded using a Luminescence Spectrophotometer LS50B (Perkin-Elmer, USA). The samples, dissolved in water (0.5 mg/ml), were subjected to excitation at 370 nm and the emission was read at a wavelength of 440 nm. Fluorescence is expressed as arbitrary intensity fluorescence units per 0.5 mg protein from a representative experiment. Each value is averaged from 3 scans.

RESULTS

Preparation of glycation products under the high pressure and their analysis

A commercial preparation of BSA was purified on a Sephadex G-200 column prior to conjugation, and only a pure homogeneous fraction of the monomer (data not shown) was used for experiments. Samples of an aqueous solution, each containing BSA and lactose (lac), were kept at 4°C under different conditions of time and pressure, namely under 300 or 400 MPa for 3 h and under 800 MPa for 1 h. After the experiment, all samples retained the form of clear solutions. Then the reaction mixtures were subjected to gel filtration chromatography on a Sephadex G-200 column. A typical chromatogram is shown in Fig. 1A. Three major fractions were distinguished (BSA-lac-1–3), which were then analyzed in the SDS-PAGE assay with 10% polyacrylamide, as shown in Fig. 1a. The yield of the fractions BSA-lac-1, BSA-lac-2, and BSA-lac-3 from the initial protein used for conjugation was 16, 12.7, and 71.2%, respectively (Table 1). The MALDI mass spectrum (not shown) suggests that the material present in the fraction BSA-lac-3 contained glycated BSA; its ion peak has an average m/z of 66,852 Da compared with the average m/z of 66,500 Da of the ion peak of the original purified BSA. Fractions BSA-lac-1 and BSA-lac-2 are cross-linked glycation products when compared with the BSA standard, as can be inferred from SDS-PAGE (Fig. 1A, a). Further experiments were performed with a mixture of ribose (rib) and myoglobin (Mb) from horse muscle, which was exposed to a pressure of 600 or 860 MPa for 4 h. Fractions Mb-rib-1 and Mb-rib-2 from

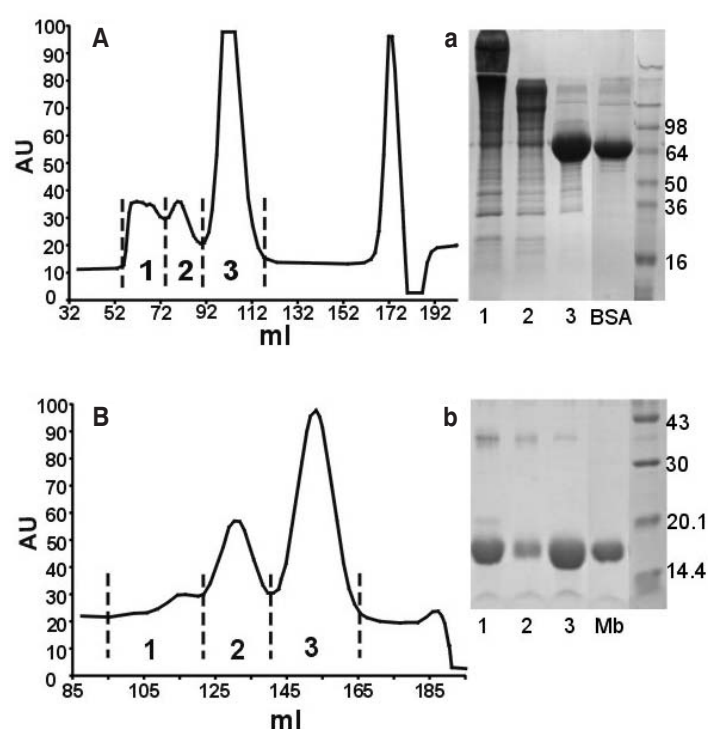


Figure 1. Fractionation on Sephadex G-200 column (1.6×100 cm) with PBS as an eluent (**A, B**) and inserted SDS-PAGE patterns stained with Coomassie Brilliant Blue R250 (**a, b**) of glycation products. **A, a** – BSA and lactose (14 h, 600 MPa); 1 – BSA-lac-1; 2 – BSA-lac-2; 3 – BSA-lac-3; **B, b** – myoglobin and ribose (4 h, 600 MPa and then 4 h, 860 MPa); 1 – Mb-rib-1; 2 – Mb-rib-2; 3 – Mb-rib-3.

Table 1. Yields of products obtained by two methods, expressed as the percentage of protein taken for synthesis

Product		Percentage of fraction						
HPG	HTG	Insoluble material	1	I	2	II	3	III
Mb-lac		0	4.8		16.5		38.3	
Mb-rib		0	17.5		8.0		36.0	
Mb-fru		0	4.0		20.0		45.5	
	Mb-lac	33.8		13.0		14.5		12.3
BSA-lac		0	16.0		12.7		71.2	
	BSA-lac	13.9		15.0		5.0		45.0
Average		0	23.9	10.6	14.3	9.8	47.8	28.7

Fractions 1, 2, and 3 were obtained by the HPG method and I, II and III were synthesized by the HTG procedure.

Sephadex G-200 (Fig. 1B) contained high- and low-molecular-mass material, as shown with 15% polyacrylamide gel in SDS-PAGE (Fig. 1b). Similar results were obtained for lactose-modified Mb (data not shown).

Comparative analysis of the products formed under high pressure and at high temperature

In order to compare the products obtained in HPG²¹ with another method of glycation, the substrates described above were subjected to HTG reaction³. In contrast to HPG carried out in water solution, the HTG is a dry reaction of lyophilized samples. Gel filtration chromatography on a Sephadex G-200 column revealed differences in the chromatographic patterns and yields of the products between the two methods.

The results are presented in Fig. 2. The fraction BSA-lac-3 (HPG) was eluted in a volume of lower molecular mass than was the fraction BSA-lac-III (HTG; Fig. 2A), which was confirmed by SDS-PAGE (10% polyacrylamide gel; Fig. 2B, lanes III, 3). In general, HPG products had a lower molecular mass than did HTG products. When an Mb-lac mixture was subjected to HPG and, for comparison, to HTG, the pattern was similar to the one observed in previous experiments with BSA: the HPG products were eluted from the Sephadex G-200 column in a larger volume than were the HTG products (Fig. 3A). The MALDI mass spectrum of fraction Mb-lac-3 (HPG) contained an ion peak corresponding to a molecular mass of 17,106 Da, that of fraction Mb-lac-III (HTG) had a peak related to a molecular mass of 19,721 Da (Fig. 3B), and that of the Mb standard showed an ion peak of m/z 16,957 Da.

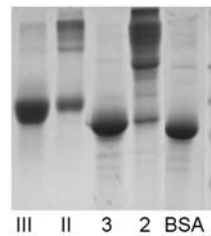
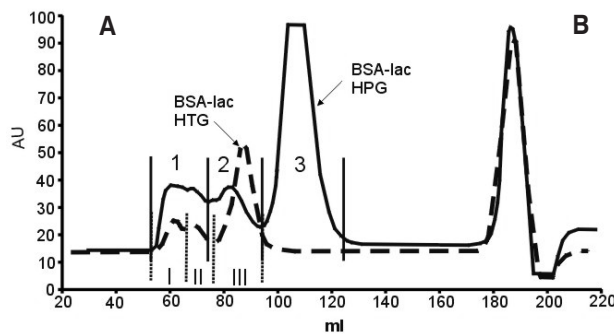


Figure 2. Comparison of BSA-lactose products obtained with two methods, high-pressure glycation (HPG) and high-temperature glycation (HTG): **A** – fractionation on Sephadex G-200 column (1.6×100 cm) of HPG products marked with the thin line (fractions 1, 2, 3) and HTG products marked with the thick line (fractions I, II, III); **B** – SDS-PAGE analysis pattern of the fractions: (HTG) BSA-lac-III (III), BSA-lac-II (II); (HPG) BSA-lac-3 (3) and BSA-lac-2 (2). Staining of electrophoretic gel with the Coomassie Brilliant Blue R250 reagent.

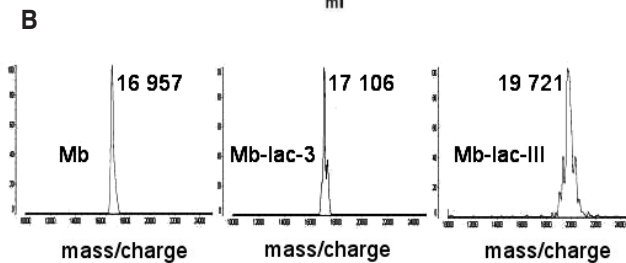
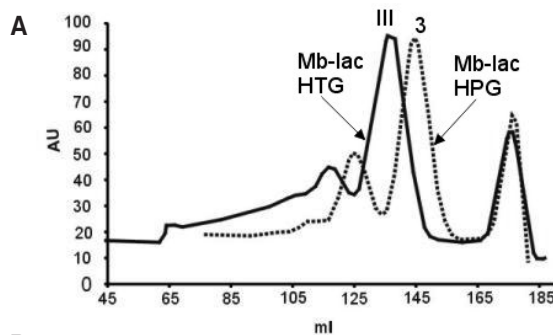


Figure 3. Comparison of myoglobin-lactose products obtained with two methods, high-pressure glycation (HPG) and high-temperature glycation (HTG): **A** – separation of HPG (thin line) and HTG (thick line) products on Sephadex G-200 column (1.6×100 cm) and PBS as an eluent. **B** – MALDI mass spectra of Mb-lac-3 (HPG), Mb-lac-III (HTG) and Mb.

The yields of the fractions obtained with both the methods are summarized in Table 1. These data indicate that the HPG method is as efficient as the HTG procedure with regard to the comparable yields of total soluble cross-linked products from the HPG (fractions 1 and 2; 24.9%) and the HTG method (fractions I and II; 23.8%). The average yield of the low-molecular-mass products from the HPG method (fraction 3; 47.8%) is higher than that from the HTG (fraction III; 28.7%), while at the same time the HTG procedure gave about 30% of insoluble material.

Analysis of the content of amino groups in the BSA-lac fractions from the HTG and HPG methods

(Table 2) indicated that the highest level of primary amino group modification was in the fractions BSA-lac-I–III and BSA-lac-2. Interestingly, fraction BSA-lac-II obtained with the HTG method, with a 77% loss in amino groups, expressed the highest intensity of fluorescence (Table 2). Generally, the fluorescent AGEs content, higher in HTG than in HPG products, was compatible with the higher level of sugar and of the modification of amino groups.

Serological study of glycation products

In order to produce polyclonal antibodies against AGE cross-linked antigens, rabbits were immunized with BSA-lac-2 (HPG), BSA-lac-III (HTG) or Mb-lac-II (HTG) products. Serological analysis based on SDS-PAGE – immunoblotting experiments (Fig. 4) revealed the following: serum anti-BSA-lac-III (HTG) reacted with Mb-lac-II (HTG; Fig. 4B, lane 2), BSA-lac-II (HTG; Fig. 4B, lane 4), and BSA-lac-III (Fig. 4B, lane 5), but there was no reaction with the corresponding HPG products (Fig. 4B, lanes 1, 6), and serum anti-BSA-lac-2 (HPG) did not react with the BSA-lac-II (HTG) products (Fig. 4D, lane 4). Similar results were obtained with serum anti-Mb-lac-II (HTG; Fig. 4C), which reacted with the HTG products and did not react with the HPG products. Antibodies against HTG products recognized epitopes on modified BSA and Mb. It is interesting to note that there was only a trace response to carrier proteins.

DISCUSSION

AGEs accumulate in several tissues during life, but their accumulation is more extensive in diabetes patients^{5–7, 22, 24–26}. It is very important to monitor the process in order to predict potential complications. The use of markers, possibly correlated (in terms of presence and concentration) with the development of the disease, seems to be well suited for this purpose. Although there are many possible products of glyca-

Table 2. The analysis of glycated proteins with chemical and spectroscopic methods

Sample			Total neutral sugar ^a (%)	IFU ^b	Free amino groups ^c (%)
HTG	HPG	other			
BSA-lac-I			3.8±0.4	34.0	20
BSA-lac-II			13.3±3.1	82.0	23
BSA-lac-III			4.4±0.6	23.0	15
	BSA-lac-1		2.8±0.9 (1.6)	19.0	54
	BSA-lac-2		1.8±0.9 (0.6)	7.0	15
	BSA-lac-3		0.8±0.6 (0.3)	11.0	65
Mb-lac-I			nt ^d	15.0	nt
Mb-lac-II			nt	14.0	nt
Mb-lac-III			nt	13.0	nt
	Mb-lac-1		nt	28.0	nt
	Mb-lac-2		nt	2.5	nt
	Mb-lac-3		nt	1.9	nt
		BSA-lac ^e	nt	25.0	84
		BSA	2.1±0.7 (0.25)	37.0	100
		Mb	nt	0.5	nt

^a Total neutral sugar determined by the phenol/sulfuric acid reagent; in parentheses are values from sugar analysis determined by the GLC-MS method.

^b Intensity of the fluorescence emission at 440 nm after excitation with a wavelength of 370 nm, expressed as the arbitrary intensity fluorescence units (IFU) per 0.5 mg protein from a representative experiment. Each value is the average of 3 scans.

^c Percentage of free amino groups in relation to the unmodified protein, average of two repeated determinations.

^d Not tested; in the case of Mb, the interference of the hem group disabled the colorimetric determinations.

^e Product obtained by spontaneous glycation in solution at 37°C for 4 weeks.

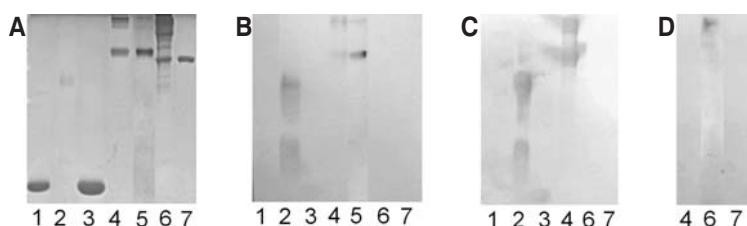


Figure 4. Serological analysis of glycation products 1 – Mb-lac-2 (HPG); 2 – Mb-lac-II (HTG); 3 – Mb; 4 – BSA-lac-II (HTG); 5 – BSA-lac-III (HTG); 6 – BSA-lac-2 (HPG); 7 – BSA. **A** – 12.5% SDS-polyacrylamide gel electrophoresis; **B** – immunoblotting with rabbit serum anti BSA-lac-III; **C** – immunoblotting with rabbit serum anti Mb-lac-II; **D** – immunoblotting with rabbit serum anti BSA-lac-2.

tion, only a few of them have been identified²⁸. It is believed that one of the best approaches to the determination of the AGEs in living tissues is immunochemistry¹. A key issue is therefore the availability of such synthetic products that can help produce specific antibodies and may also be used as molecular diagnostic probes. A known procedure for such synthesis includes incubation of an aqueous mixture of protein and sugar at 37°C for several days^{27, 29}, which is extremely time consuming. Much shorter is the heating of a lyophilized mixture of substrates³, where the early glycation products are efficiently formed. When AGEs are to be used as markers, the HPG process may be used as an alternative (Table 1). This has two major advantages: the yield of cross-linked AGEs is good and the time of the reaction can be reduced to only a few hours. In the one-step reaction without chemical reagents added, the obtained products were soluble and highly cross-linked, possessing distinct serological properties. In the advanced products, the protein molecules were cross-linked by modified sug-

ars and could still be depolymerized by pronase (data not shown). The HPG process involves substrates in aqueous solution and temperatures below 25°C, which prevents protein damage. The HPG procedure is preferred also when advanced products are needed from defined small substrate molecules which are volatile or hygroscopic and cannot be lyophilized, such as methylglyoxal. This method is milder than the HTG process and stimulates the formation of soluble cross-linked material; in the standard HTG procedure, about 30% of the protein obtained is insoluble (Table 1).

The chemical characterization of fractions obtained from gel filtration chromatography revealed that the highest content of fluorescent AGEs was in the HTG fraction with intermediate molecular mass (fraction BSA-lac-II). The total neutral sugar determination seems not to be reliable for analysis of glycation due to the possibility of interference of color from glycation products.

A key issue in the synthesis of the glycation model adducts is the appropriate choice not only of the sugar, but also of the protein. Commercial proteins should be checked for sugar contaminants. It has been previously reported that commercial preparations of BSA are naturally glycated¹⁹. We also observed in our preliminary experiments that BSA originally contained some modified molecules, as revealed by SDS-PAGE (data not shown). GLC-MS analysis of alditol acetates disclosed the presence of man, glc, and gal in molar ratios of 1:1.6:1, respectively, in our native preparation of BSA. Even when the commercial BSA was separated on the Sephadex G-200 column, the residual sugar participated in the glycation reaction. Unlike BSA, the preparations of Mb were free of carbohydrate contaminants.

The conditions of glycation in solution are known to influence the reactivity of the substrate and the extent of degradation¹⁷. In our experiments, the sera specific to glycation products formed under different methods did not show cross-reactivity. Different conditions of glycation created various epitopes. Further experiments with HPG products as markers for the analysis of tissue samples are underway. Remarkably,

high hydrostatic pressure yielded products which differed from those of the reaction of a dry mixture of a lyophilized material at high temperature. However, it is worth noting that the HPG procedure has more advantages than does the related, commonly used spontaneous glycation in solution, which takes as long as several weeks. The comparison of the two procedures of glycation presented here suggests that they may be regarded as being complementary.

In summary, the conditions of HPG of proteins have been established and, compared with HTG, the method gives entirely soluble cross-linked products. We observed that, as evidenced from decreased amino groups and increased fluorescent AGEs content, the products formed with these two methods are different, presumably in that fluorescent products are formed by the HTG method. Interestingly, the fluorescent AGEs have intermediate molecular mass. The most relevant observation is that quite different epitopes are formed under the conditions of these two methods of HTG and HPG. Further experiments are planned to verify the possible use of AGEs produced to study glycation-related pathologies.

REFERENCES

- Al-Abed Y. and Bucala R. (2000): Structure of a synthetic glucose derived advanced glycation end product that is immunologically cross-reactive with its naturally occurring counterparts. *Bioconjug. Chem.*, 11, 39–45.
- Barciszewski J., Jurczak J., Porowski S., Specht T. and Erdmann V. (1999): The role of water structure in conformational changes of nucleic acids in ambient and high-pressure conditions. *Eur. J. Biochem.*, 260, 293–307.
- Boratynski J. and Roy R. (1998): High temperature conjugation of proteins with carbohydrates. *Glycoconj. J.*, 15, 131–138.
- Bourdon E., Loreau N. and Blache D. (1999): Glucose and free radicals impair the antioxidant properties of serum albumin. *FASEB J.*, 13, 233–244.
- Brownlee M. (2001): Biochemistry and molecular cell biology of diabetic complications. *Nature*, 414, 813–820.
- Cai J. and Hurst H. E. (1999): Identification and quantitation of N-(carboxymethyl)valine adduct in hemoglobin by gas chromatography/mass spectrometry. *J. Mass Spectrom.*, 34, 537–543.
- Cheng R., Lin B. and Ortwirth B. J. (2003): Separation of the yellow chromophores in individual brunescant cataracts. *Exp. Eye Res.*, 77, 313–325.
- Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A. and Smith F. (1956): Colorimetric method for determination of sugar and related substances. *Anal. Chem.*, 28, 350–356.
- Gamian A., Kenne L., Mieszała M., Ulrich J. and Defaye J. (1994): Structure of the *Escherichia coli* O24 and O56 O-specific sialic-acid-containing polysaccharides and linkage of these structures to the core region in lipopolysaccharides. *Eur. J. Biochem.*, 225, 1211–1220.
- Gamian A., Romanowska A. and Romanowska E. (1992): Immunochemical studies on sialic acid-containing lipopolysaccharides from enterobacterial species. *FEMS Microbiol. Immunol.*, 4, 323–328.
- Girard M., Cyr T., Mousseau N. and Ethier J. C. (1999): Separation of human serum albumin components by RP-HPLC and CZE and their characterization by ESI-MS. *Chromatographia*, 49 (suppl. 1), 21–27.
- Hamley R. J. (1995): Turning off the water. *Nature*, 378, 14–15.
- Hudson B. I., Bucciarelli L. G., Wendt T., Sakaguchi T., Lalla E., Qu W., Lu Y., Lee L., Stern D. M., Naka Y., Ramasamy R., Yan S.D., Yan S.F., D'Agati V. and Schmidt A. M. (2003): Blockade of receptor for advanced glycation endproducts: a new target for therapeutic intervention in diabetic complications and inflammatory disorders. *Arch. Biochem. Biophys.*, 419, 80–88.
- Kao G. L. and Tsai C. M. (1997): Detection of lipopolysaccharides electroblotted onto membranes. *J. Endotoxin Res.*, 4, 459–462.
- Laemmli U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680–685.
- Lee Y. C. (1978): Synthesis of some cluster glycosides suitable for attachment to proteins or solid matrices. *Carbohydr. Res.*, 67, 509–514.
- Lo T. W., Westwood M. E., McLellan A. C., Selwood T. and Thornalley P. J. (1994): Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with N α -acetylarginine, N α -acetylcysteine, and N α -acetyllysine, and bovine serum albumin. *J. Biol. Chem.*, 269, 32299–32305.
- Lubineau A., Auge J. and Queneau Y. (1994): Water-promoted organic reactions. *Synthesis*, 741–760.
- Lucey M. D., Newkirk M. M., Neville C. and Lepage K. (2000): P.R. Fortin, Association between IgM response to IgG damaged by glyoxidation and disease activity in rheumatoid arthritis. *J. Rheumatol.*, 27, 319–323.
- Lugowski C., Kulakowska M. and Romanowska E. (1986): Characterization and diagnostic application of a lipopolysaccharide core oligosaccharide-protein conjugate. *J. Immunol. Methods*, 95, 187–194.

21. Moreno F. J., Molina E., Olano A. and Lopez-Fandino R. (2003): High-pressure effects on Maillard reaction between glucose and lysine. *J. Agric. Food Chem.*, 51, 394–400.
22. Newkirk M. M., Goldbach-Mansky R., Lee J., Hoxworth J., McCoy A., Yarboro C., Klippel J. and El-Gabalawy H. S. (2003): Advanced glycation end-product (AGE)-damaged IgG and IgM autoantibodies to IgG-AGE in patients with early synovitis. *Arthritis Res. Ther.*, 5, R82–90.
23. Oya T., Hattori N., Mizuno Y., Miyata S., Maeda S., Osawa T. and Uchida K. (1999): Methylglyoxal modification of protein. Chemical and immunochemical characterization of methylglyoxal-arginine adducts. *J. Biol. Chem.*, 274, 18492–18502.
24. Sell D. R. and Monnier V. M. (1989): Structure elucidation of a senescence cross-link from human extracellular matrix. Implication of pentoses in the aging process. *J. Biol. Chem.*, 264, 21597–21602.
25. Staniszevska M. and Gamian A. (2003): Biochemical properties and clinical significance of protein glycation products. *Postepy Hig. Med. Dosw.*, 57, 123–147.
26. Swamy-Mruthinti S., Miriam K. C., Kumar S. K., Biswas J., Ramakrishnan S., Nagaraj R. H. and Sulochana K. N. (2002): Immunolocalization and quantification of advanced glycation end products in retinal neovascular membranes and serum: a possible role in ocular neovascularization. *Curr. Eye Res.*, 25, 139–145.
27. Tagami U., Akashi S., Mizukoshi T., Suzuki E. and Hirayama K. (2000): Structural studies of the Maillard reaction products of a protein using ion trap mass spectrometry. *J. Mass Spectrom.*, 35, 131–138.
28. Thorpe S. R. and Baynes J. W. (2003): Maillard reaction products in tissue proteins: new products and new perspectives. *Amino Acids*, 25, 275–281.
29. Voziyan P. A., Khalifah R.G., Thibaudeau C., Yildiz A., Jacob J., Serianni A. S. and Hudson B. G. (2003): Modification of proteins *in vitro* by physiological levels of glucose: pyridoxamine inhibits conversion of Amadori intermediate to advanced glycation end-products through binding of redox metal ions. *J. Biol. Chem.*, 278, 46616–46624.