Structural stability of myoglobin and glycomyoglobin: a comparative molecular dynamics simulation study

Joulia Alizadeh-Rahrovi · Alireza Shayesteh ·
Azadeh Ebrahim-Habibi

Received: 30 October 2014 / Accepted: 26 January 2015 / Published online: 21 February 2015
© Springer Science+Business Media Dordrecht 2015

Abstract Glycoproteins are formed as the result of enzymatic glycosylation or chemical glycation in the body, and produced in vitro in industrial processes. The covalently attached carbohydrate molecule(s) confer new properties to the protein, including modified stability. In the present study, the structural stability of a glycoprotein form of myoglobin, bearing a glucose unit in the N-terminus, has been compared with its native form by the use of molecular dynamics simulation. Both structures were subjected to temperatures of 300 and 500 K in an aqueous environment for 10 ns. Changes in secondary structures and RMSD were then assessed. An overall higher stability was detected for glycomyoglobin, for which the most stable segments/residues were highlighted and compared with the native form. The simple addition of a covalently bound glucose is suggested to exert its stabilizing effect via increased contacts with surrounding water molecules, as well as a different pattern of interactions with neighbor residues.

Keywords Myoglobin · Glycomyoglobin · Molecular dynamics simulation · Structural stability · Glucose

Electronic supplementary material The online version of this article (doi:10.1007/s10867-015-9383-2) contains supplementary material, which is available to authorized users.

J. Alizadeh-Rahrovi · A. Shayesteh (✉)
School of Chemistry, College of Science, University of Tehran, Tehran, Iran
e-mail: ashayesteh@ut.ac.ir

A. Ebrahim-Habibi (✉)
Biosensor Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran
University of Medical Sciences, Shariati Hospital, North Kargar Avenue, 1411413137 Tehran, Iran
e-mail: aehabibi@sina.tums.ac.ir

Azadeh Ebrahim-Habibi
e-mail: azadehabibi@yahoo.fr

A. Ebrahim-Habibi
Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences
Institute, Tehran University of Medical Sciences, Tehran, Iran

1 Introduction

Ligand binding may affect the structure and properties of proteins and is particularly important in the case of biologically important proteins such as the globin family [1]. Globins possess mostly alpha-helical folds, contain a heme prosthetic group, and include myoglobin (Mb) and hemoglobin (Hb). These biomolecules perform a range of functions, from oxygen storage to redox reactions and catalysis, with the use of their “active site” that contains the heme group and its local environment [2, 3].

Myoglobin is a monomeric heme-containing protein located in muscle tissues, that binds and releases O₂ and small ligands such as CO via its heme residue [4, 5]. It is related to hemoglobin, which is the oxygen transport protein in blood, and specifically in the red blood cells [6]. Myoglobin’s three-dimensional structure consists of eight α -helices connected by loops, which are packed against each other and encompass the heme group [7]. Coordinative bonds to the iron atom (located in the center of heme prosthetic group), are essential for maintaining the structure and oxygen-binding function of the protein [8].

The addition of carbohydrate molecules to free amine groups of protein residues is one of the important so-called post-translational modifications [9]. Targeted, enzyme-mediated addition of carbohydrates is usually functionally essential [9], while chemical glycation of the proteins may have detrimental effects on their structure and function [10, 11]. It is interesting to note that glycosylation of industrially important enzymes may actually be essential for their stability, as well as their expression level in the host organism [12].

Glycation of albumin and hemoglobin may happen as a result of increased glucose concentration in the body, and is used as a marker of diabetes mellitus [13]. In the normal body, advanced glycated end products (AGEs) form at a slow rate, while in diabetes their formation accelerates due to chronically high concentration of blood sugar, which enhances the availability of glucose [14]. AGEs damage cells by affecting the structure and function of proteins [15] that can persist for a while in the long-lived cells such as brain and nerve cells and DNA. They are involved in many age-related diseases such as type 2 diabetes mellitus, Alzheimer’s disease, cancer, and cardiovascular diseases [11, 16, 17].

The N-terminus residue of the Mb sequence, namely glycine, is susceptible to glycation, since free amino groups are able to react with and bind to glucose [9]. The present study aimed at comparing the structure and stability of a glucose-bound form of Mb (GMb) with the native form, with the use of molecular dynamics (MD) simulations at two different temperatures.

2 Methods

2.1 Protein structure

The structure that was used in the current study is a high-resolution structure of human Mb (1.65 Å) that has been deposited in the Protein Data Bank with the PDB code of 3RGK. This is so far the only high-resolution X-ray-solved variant of human Mb. The last four residues of the structure (Gly150–Gly153) backbones were corrected prior to simulation with the use of MOE.2010.10 (Molecular Operating Environment, Chemical Computing Group Inc., Montreal, Canada). Additional molecules to protein were deleted. In order to make GMb, a glucose molecule was added to the N-terminus of Mb with the use of the build module of MOE.2010.10. The protein protonation states were adjusted at pH=7.14 using the biopolymer module of MOE.2010.10, positioned into a periodic box, and explicit rigid water molecules were added as solvent.

2.2 Simulation

Simulation was done with MOE.2010.10 using the MMFF94x force field. The system was first minimized until the root mean square gradient fell below 1. Simulations were performed using the Nosé–Poincaré–Andersen Hamiltonian equation of motion (NPA) algorithm, with temperatures of 300 K and 500 K on both Mb and GMb. First, the systems were subjected to a 100-ps equilibration step after which a 10-ns MDS was performed for each system. An analysis of the output data with regard to the secondary structure of the proteins (topology) was performed by Pro-origami [18], which is available at <http://munk.csse.unimelb.edu.au/pro-origami/> and the DSSP chart was prepared with the use of a DSSP web server (<http://www.cmbi.ru.nl/dssp.html>).

3 Results and discussion

Potential energy plots of the systems show that the systems have achieved convergence over the 10-ns simulation time (Fig. 1).

The conformational differences of the models were analyzed in order to assess their structural stability. The analysis was performed by comparing systems' root-mean-square deviation (RMSD) [19].

The RMSD of sampled structures' coordinates relative to the initial model are displayed in Fig. 2, as a function of time. These structures have been sampled every 100 ps, and used throughout the study. The RMSDs of both systems do not show any major change at 300 K. The overall structure of the protein chains almost matches that of the crystallographic model: final average RMSD values were 0.97 Å and 0.95 Å for Mb and GMb, respectively. In order to further explore the conformational stability of the protein, RMSD was followed at a higher temperature (500 K) to achieve a broader range of structural changes. According to the plots of Fig. 2, GMb shows lower RMSD values on the whole (average of 4.40 Å compared with average of 5.08 Å for Mb) which is an indication of higher stability. In both systems though, an unfailing increase in the RMSDs at 500 K indicates the continuous evolving of structures during simulation time at 500 K. The protein structures are in fact unfolded during the simulation period (Fig. S1). However, some residues of the final structures have still remained

Fig. 1 Evolution of potential energy during the simulation time intervals for native Mb at 500 K (a), glycomyoglobin at 500 K (b), native Mb at 300 K (c), and glycomyoglobin at 300 K (d)

Fig. 2 Evolution of RMSDs during 10-ns simulation of Mb and GMb at 300 K and 500 K with respect to the minimized crystal structure. Native Mb at 500 K (a), glycomyoglobin at 500 K (b), native Mb at 300 K (c), and glycomyoglobin at 300 K (d)

in their original helical fold. These may be hypothesized to be the more stable structural segments of the proteins [20].

In order to have a closer look at these segments, an RMSD plot of all single residues was prepared by manual superposition of the 100 sampled structures over time (Fig. 3). Values that are higher than corresponding overall RMSD, i.e., 0.58 Å and 0.59 Å for Mb and GMb at 300 K and 3.42 Å, and 3.30 Å for Mb and GMb at 500 K, are colored in red. Hence, areas with high or low changes could be spotted: the colorless cells show the segments with minor conformational changes and therefore major stability. It can be inferred from the plots that

Fig. 3 RMSD of all residues of Mb in the 100 sampled structures over time. Darker (red) areas are related to RMSD values that are higher than the overall RMSD for native Mb at 300 K (a), glycomyoglobin at 300 K (b), native Mb at 500 K (c), and glycomyoglobin at 500 K (d)

these areas are somewhat different for the systems at different temperatures. Since these are more distinct at 500 K, residues that remain stable at this temperature have been more specifically shown in Fig. 4. Interestingly, residues that are closer to the N-terminus experience less dramatic changes in Gmb compared to the native form. Most stable regions in Mb are Gly1-Trp7, Trp7-Gly23 (that remain stable for a longer time compared with the 1–7 segment), Leu32-Pro37, Gly74-Gly80, Ala94-Val101, Val114-Ala125, Ala125-Leu137, Met142-Tyr146, and Asn145-Tyr146. In Gmb, these are Gly1-Trp7, Trp7-Leu32, Leu32-Leu40, Gly74-Gly80, Ile86-Lys96, Pro100-Ile107, His119-Gly124, and Gly124-Tyr146. The Gly1 to Trp7 segment is common between Mb and Gmb, but shows markedly higher stability over time in Gmb. This is also the case for Gly74 to Gly80. The area Leu32 to Pro37 in Mb and Leu32 to Leu40 (a broader segment) in Gmb also remain stable for a relatively long time.

Based on the previous section's data, the residues that are close to glucose seem to be of importance in the observed increased stability of Gmb. Accordingly, the amino acids located at a distance of 4.5 Å from Gly1 were selected in Gmb and their RMSD changes were followed over time. Figure 5 shows the RMSD of these residues: Leu2, Ser3, Lys140, Asp141, Leu137, and Glu136. Leu2 and Ser3 have the lowest fluctuation in RMSD and could be stated to be the most stable amino acids in this selection. On the other hand, Leu2 is the only residue to be covalently linked to Gly1. The other residues are also more stable in Gmb than their Mb

Fig. 4 Highlights of residues that undergo fewer changes and remain stable at 500 K in simulations of native (a) and glycosylated (b) forms of myoglobin

Fig. 5 RMSD of amino acids located at 4.5 Å of Gly1, Leu2 (a), Ser3 (b), Lys140 (c), Asp141 (d), Leu137 (e), and Glu136 (f)

counterparts in the earlier time period of the simulation, with the exception of Glu136, which is also located somewhat farther than other residues and does not seem to make any significant interaction with the glucose molecule. Hydrogen bonds formed between Gly1-adjacent residues and water molecules have been compared in ten sampled Mb and GMb structures (Tables 1 and 2 Supplementary Fig. S2). From this data, different interaction partners are detected for Asp141 and Lys140. In the Mb snapshots, Asp141 is close to Gly1, while in GMb, it is within hydrogen bond range with Lys140. Three representative structures (taken at the start, in the middle, and at final simulation time) are reported in Fig. 6. The Lys140-Asp141 pair (which

Fig. 5 (continued)

could also be considered as a salt bridge) remains constant over time in Gmb, whereas in Mb, due to Asp141 interaction with Gly1, Lys140 is forms a hydrogen bond with Glu136. Looking at Gmb snapshots, Glu136 is found to be in contact with Arg139, while in Mb, the Arg139 side chain moves away and makes contact with backbone oxygens of adjacent chains (not shown). Overall, such kind of disruption that happens in residue interactions in Mb may have a role in the changes that occur in the integrity of helices. It should also be pointed out that the presence of glucose in Gmb favors a higher number of contacts with water molecules at the N-terminus of the modified protein, which could be relevant in its stabilization.

Table 1 Detailed interactions (hydrogen bonds) between N-terminal residue of myoglobin, Gly1, at the temperature of 500 K, and its nearby residues (4.5 Å) in ten sampled structures

Mb_500K			
Time (ns)	Selected residue	Nearby (4.5 Å) residues	Res-Res interaction
0	Gly1	Leu2 Ser3 Leu137	Gly1-WM2449 WM2729 WM2780 Leu2-WM2790
100	GLy1	Leu2 Ser3 Lys133 Glu136	Gly1-WM399 WM506 WM1288 WM2492 Leu2-WM1955 WM2939 Lys133-WM399 WM2492 Glu136-WM2492 WM2810
200	Gly1	Leu2 Ser3 Lys133 Glu136	Gly1-WM102 WM506 WM3017 WM4980 Leu2-WM298 WM3119 Lys133-WM102 WM4980 Glu136-WM506 WM2204 WM2454
300	Gly1	Leu2 Ser3 Lys133 Lys137	Gly1-WM3079 WM3756 WM4286 WM4990 Leu2-WM570 Lys133-WM2330
400	Gly1	Leu2 Ser3 Glu6 Lys133	Gly1-WM3778 WM4223 WM4253 Lys133 Leu2-WM906 Ser3-WM2131 Glu6 Glu6-Ser3 Lys133-WM2131 Gly1
500	Gly1	Leu2 Ser3 Glu6 Lys133 Lys137 Asp141 Met143	Gly1-WM2675 WM2724 WM3894 Lys133 Leu2-WM5398 WM5519 Ser3-Glu6 Glu6-Ser3 Lys133 Lys133-Glu6 WM2848

Table 1 (continued)

Mb_500K

Time (ns)	Selected residue	Nearby (4.5 Å) residues	Res-Res interaction
600	Gly1	Leu2 Lys133 Leu137 Asp141	Asp141-1486 WM1966 WM2724 WM3150 WM3894 WM4922
			Gly1-WM1966 WM2446 WM2506 Asp141 Leu2-WM3142 Lys133-WM2506 Asp141-WM235 WM1372 Gly1
			Gly1-WM4052 WM1699 Lys133 Asp141 Leu2-WM30 WM423 Ser3-WM3413 Glu6 Glu-Ser3 Lys133 Lys133-WM3413 Gly1 Glu6 Glu136-WM1226 WM1699 WM4886 Asp141-WM2548 WM5388 WM3399 Gly1
			Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1
700	Gly1	Leu2 Ser3 Glu6 Lys133 Glu136 Leu137 Asp141	Gly1-WM4052 WM1699 Lys133 Asp141 Leu2-WM30 WM423 Ser3-WM3413 Glu6 Glu-Ser3 Lys133 Lys133-WM3413 Gly1 Glu6 Glu136-WM1226 WM1699 WM4886 Asp141-WM2548 WM5388 WM3399 Gly1
			Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1
			Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1
			Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1
800	Gly1	Leu2 Ser3 Glu6 Lys133 Leu137 Asp141	Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1
			Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1
			Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1
			Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1
900	Gly1	Leu2 Ser3 Glu6 Lys133	Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1
			Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1
			Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1
			Gly1-WM65 WM698 Asp141 Lys133 Leu2-WM65 WM1699 Ser3-WM3169 WM3574 Lys133-WM3353 Gly1 Asp141-WM3667 WM3976 WM4611 Gly1

Table 1 (continued)

Mb_500K

Time (ns)	Selected residue	Nearby (4.5 Å) residues	Res-Res interaction
1000	Gly1	Asp141	Leu2-WM65 WM1653 WM3596 Glu6-WM4219 Lys133 Lys133-WM3353 Gly1 Asp141-WM877 WM3150 WM3964 Gly1
		Leu2 Ser3 Lys133 Lys140 Asp141	Gly1-WM1664 WM3964 WM2729 Ser3 Asp141 Leu2-Asp141 Ser3-WM73 Gly1 Lys133-WM1664 WM2697 Asp141-WM2951 WM4418 Gly1 Leu2

Hyphens mean “interacts with”. The term “WM” is the abbreviation for water molecule

The changes that happen in the secondary structure of Mb and GMb over time have been represented with DSSP charts (Fig. 7). These charts include 21 snapshots of protein secondary structures and it could be observed that, at 300 K, both systems are relatively stable. At 500 K, alpha-helix content gradually decreases, which happens more rapidly for Mb (Fig. 7c) compared to GMb (Fig. 7d). Averages of water-accessible surface area (ASA) and radius of gyration (Rgyr) of systems were also calculated during the MDS time intervals. Average values of Mb ASA were 33,198.25 Å² and 32,178.95 Å², and were 34,191.68 Å² and 32,848.49 Å² for GMb at 300 and 500 K, respectively. Average values of Mb Rgyr were 29.00 Å and 29.22 Å and were 29.24 Å and 29.61 Å for GMb at 300 K and 500 K, respectively. Overall, these values are close to each other, and the difference between Mb and GMb may be related to the presence of the glucose moiety in GMb. Finally, the overall secondary structures of Mb and GMb were compared with each other, with the use of the last output structure of the 500 K simulation (Fig. 8). Mb was found to possess three helices, while GMb showed 13 helices, which are shorter than the original structures. If alpha-helices are defined as containing at least four residues, eight acceptable helices would be detected in GMb.

Although the closest residues to glucose were actually much influenced by its presence, both RMSD data and secondary structure analysis show that GMb structure has been stabilized beyond the immediate vicinity of its N-terminus. Similar observations have been made in the case of glycosylated proteins in experimental studies, highlighting the long-range effect of glycan binding [21]. The stabilizing effect of covalently attached carbohydrate molecules has

Table 2 Detailed interactions (hydrogen bonds) between N-terminal modified residue of glycomyoglobin (Gly1 and attached glucose molecule), at the temperature of 500 K, and its nearby residues (4.5 Å) in ten sampled structures

GMb_500K			
Time	Selected residue	Near (4.5 Å) residues	Res-Res interaction
0	Gly1+Glc	Leu2 Ser3 Glu6 Lys133 Leu137 Lys140	Glc-Glc WM676 WM3026 WM2953 WM2847(2) WM2752 WM2887 Gly1 Gly1-Glc WM3044 Lys133 Ser3-Glu6(2) Glu6-WM2979 Ser3(2) Lys133 Lys133-WM2583 Gly1 Glu6 Lys140-WM1416
100	Gly1+Glc	Leu2 Ser3 GLu6 Lys133 Lys140	Glc-Glc(3) WM1501 WM4126 WM2976 WM2875 WM2839 WM759 Gly1 Gly1-Glc WM272 WM3473 Leu2-WM4652(2) Ser3-680 Glu6-3623 Ser3 Lys133 Lys133-WM272 Glu6 Lys140-WM514 WM1564
200	Gly1+Glc	Leu2 Ser3 Lys133 Glu136 Leu137 Lys140 Asp141	Glc-Glc WM329 WM3452 WM2034 WM3074 WM4393 Gly1 Gly1-WM4272 WM1215 Glc Lys133-WM4272 Glu136-WM2890 Lys140-WM1002

Table 2 (continued)

GMB_500K

Time	Selected residue	Near (4.5 Å) residues	Res-Res interaction
300	Gly1+Glc	Leu2 Ser3 Lys133 Glu136 Leu137 Lys140	Asp141
			Asp141-Lys140
			Glc-Glc
			WM3162
			WM2052
			WM5852
			WM4322
			Gly1
			Leu2
			Gly1-WM15
			WM1622
			WM2392
			Glc
			Leu2-WM1213
			WM4865
			Lys133-WM1622
			Glu136-WM593
			WM2989
400	Gly1+Glc	Leu2 Ser3 Glu136 Leu137 Lys140	WM783
			Lys140-WM349
			Glc-Glc
			WM341
			WM946
			WM2284
			Gly1
			Gly1-WM239
			WM3228
			Glc
			Leu2-WM4123
500	Gly1+Glc	Leu2 Trp7 Lys133 Glu136 Leu137 Lys140	Glu136-WM171
			WM3241
			Glc-WM229
			WM3174
			WM3249
			WM3663
			WM2834
			Gly1
			Gly1-WM958
			WM3675
			Glc
			Leu2-Trp7
			Trp7-WM868
			Leu2
600	Gly1+Glc	Leu2 Lys140	Lys133-WM357
			Glu136-WM864(2)
			Lys140-WM2039
			WM4768
			Glc-Glc(2)
			WM5758
			WM4968
			WM5349
			WM168
			Gly1

Table 2 (continued)

GMB_500K

Time	Selected residue	Near (4.5 Å) residues	Res-Res interaction
700	Gly1+Glc	Leu2 Ser3 Lys140	Gly1-WM4146
			Glc
			Leu2-WM57
			Lys140-WM2274
			WM1487
			Glc-Glc
			WM634
			WM2987
			WM5295
			WM1757(2)
			WM3243
			WM3973
			WM1528
			Gly1
800	Gly1+Glc	Leu2 Ser3 Leu137 Lys140	Gly1-WM2226
			WM3323
			Glc
			Leu2-WM4829
			Lys140-WM2795
			WM4733
			Glc-WM1456
			WM4582
			WM1613
			WM3534
			WM2960
			WM2500
			WM4201
			Gly1
900	Gly1+Glc	Leu2 Ser3 Glu6 Lys140	Gly1-WM1146
			WM3323
			Glc
			Leu2-WM3846
			Ser3-WM3150
			Lys140-WM5676
			Glc-WM1712
			WM2010
			WM2084
			WM2795
			WM3196
			WM3584
			WM3719
			WM4867
1000	Gly1+Glc	Leu2 Ser3 Glu136 Leu137	Gly1-WM2583
			WM3348
			Leu2-WM1636
			Ser3-Glu6
			Glu6-WM3348
			Ser3
			Lys140-WM1470
			Glc-WM2694
			WM3276
			WM4490
			Gly1-WM1649

Table 2 (continued)

Gmb_500K

Time	Selected residue	Near (4.5 Å) residues	Res-Res interaction
		Lys140 Asp141	WM689 WM2112 WM2974 WM4490 WM5357 Lys140 Asp141 Leu2-WM2554(2) Ser3-WM5461 Glu136-WM5357 Lys140-WM3081 Glc Asp141 Asp141-WM2366 WM3776 Lys140 Glc

Hypens mean “interacts with”. The term “*WM*” is the abbreviation for water molecule. The *numbers in parentheses* show the number of detected hydrogen bonds that occur with the corresponding residue

Fig. 6 The interactions between N-terminal residue of myoglobin (Gly1) and glycomyoglobin (Gly1 and glucose molecule) at the temperature of 500 K, and their nearby residues (4.5 Å) at three time steps (0, 5, and 10 ns) for Mb (a) and Gmb (b). Critical residues Glu136, Arg 139, Lys140, and Asp141 are shown with *sticks* while the other residues are represented with *lines*

Fig. 7 The DSSP chart of 21 structures during the MDS time interval refer to native Mb at 300 K (a), glycomyoglobin at 300 K (b), native Mb at 500 K (c), and glycomyoglobin at 500 K (d). Other structures refer to beta bridge, strand, turn and bend

been attributed to an increase in the rigidity of the glycosylated protein, as well as a decrease in surface hydrophobicity [22]. In fact, the higher thermodynamic stability of the glycosylated form compared with the deglycosylated protein has been related to a stabilization of the native state with a decrease in entropy [23]. On the other hand, there are few experimental (in vitro) reports on the effect of the glycation process on myoglobin stability. In a general manner, the non-enzymatic glycation process happens principally at the N-terminal of proteins, as well as amino side chains of lysine residues, and has also been reported to occur on arginine and histidine residues [24]. Glycation consequences vary depending on the nature of the protein that has been modified and the carbohydrate molecule: reports exist on complete inactivation of an enzyme (ribonuclease) [25], and increase of catalytic activity (hemoglobin and myoglobin esterase activity) [26]. In the case of ribonuclease, active site residues have been modified [25], while several possibilities have been postulated concerning the heme-containing proteins, including structural changes and direct effect of glucose molecules on activity-related residues [26]. Myoglobin glycation has been reported to decrease its stability alongside the increase in its structure flexibility [26], while another study reports that incubating glucose with

Fig. 7 (continued)

Fig. 8 Remaining helices of proteins after simulation time intervals at 500 K as detected by Pro-origami, three helices of native myoglobin (a) and 13 helices of Gmb (b)

apomyoglobin would not result in an increase of fibril formation in the protein (in comparison with ribose, which has the opposite effect) [27]. However, several points should be taken into consideration in this regard: first of all, glycation is a process that includes the three stages of Schiff base formation, Amadori rearrangement, and final “AGE” (advanced glycation end products) generation, with the latest being irreversible cross-linked structures [24]. It is assumed that most of the experimental works that have been done on myoglobin concern the Amadori products [28], while the species that is modeled here would be closer to the first stage of glycation [29]. Thereafter, it has been reported that in the experimental setting, which is an average-time incubation (6 days) of the protein with glucose, release of iron occurs from the heme component [28], which may potentially lead to its destruction [30], and could be one cause of glycated myoglobin instability: apomyoglobin has lower stability than holomyoglobin [31]. There is also the fact that the present work has been performed on human myoglobin, while the experimental works have been performed on either horse or whale myoglobins [27, 28] and the study that reports a lack of glucose effect on increase of myoglobin amyloid formation (in contrast to ribose), has been made on a mutated species of whale apomyoglobin [27]. Glycation effect on structure and activity of proteins would also depend on their incubation time with the carbohydrate molecule, as demonstrated in the case of aspartate aminotransferase whose activity amount differs upon brief or long incubating times with fructose [26]. Individual structural variations are important too, as hemoglobin and myoglobin exposure to similar modifiers have different results [32]. In summary, between the above-discussed experimental reports, none would be an exact equivalent of the model used in this study, which has shown an increase in the stability of a human holomyoglobin which bears a glucose moiety in the N-terminal, and could be related to a very early stage of glycation.

Acknowledgments This study was supported by the Endocrinology and Metabolism Research Institute and performed in its Modeling and Simulation Lab.

Conflict of interest The authors declare that they have no conflicts of interest to disclose.

References

1. Capece, L., Boechi, L., Perissinotti, L.L., Arroyo-Manez, P., Bikiel, D.E., Smulevich, G., Marti, M.A., Estrin, D.A.: Small ligand-globin interactions: reviewing lessons derived from computer simulation. *Biochim. Biophys. Acta* **1834**, 1722–1738 (2013)
2. Vinogradov, S.N., Hoogewijs, D., Bailly, X., Arredondo-Peter, R., Gough, J., Dewilde, S., Moens, L., Vanfleteren, J.R.: A phylogenomic profile of globins. *BMC Evol. Biol.* **6**, 31 (2006)
3. Merlino, A., Vergara, A., Sica, F., Aschi, M., Amadei, A., Di Nola, A., Mazzarella, L.: Free-energy profile for CO binding to separated chains of human and *Trematomus newnesi* hemoglobin: insights from molecular dynamics simulations and perturbed matrix method. *J. Phys. Chem. B* **114**, 7002–7008 (2010)
4. Ordway, G.A., Garry, D.J.: Myoglobin: an essential hemoprotein in striated muscle. *J. Exp. Biol.* **207**, 3441–3446 (2004)
5. Chu, K., Vojtechovsky, J., McMahon, B.H., Sweet, R.M., Berendzen, J., Schlichting, I.: Structure of a ligand-binding intermediate in wild-type carbonmonoxy myoglobin. *Nature* **403**, 921–923 (2000)
6. Ganz, T., Nemeth, E.: Regulation of iron acquisition and iron distribution in mammals. *Biochim. Biophys. Acta* **1763**, 690–699 (2006)
7. Kendrew, J.C., Bodo, G., Dintzis, H.M., Parrish, R., Wyckoff, H., Phillips, D.: A three-dimensional model of the myoglobin molecule obtained by X-ray analysis. *Nature* **181**, 662–666 (1958)

8. Stadler, A.M., Pellegrini, E., Johnson, M., Fitter, J., Zaccari, G.: Dynamics-stability relationships in apo- and holomyoglobin: a combined neutron scattering and molecular dynamics simulations study. *Biophys. J.* **102**, 351–359 (2012)
9. Han, K.K., Martinage, A.: Post-translational chemical modification(s) of proteins. *Int. J. Biochem.* **24**, 19–28 (1992)
10. Anguizola, J., Matsuda, R., Barnaby, O.S., Hoy, K.S., Wa, C., DeBolt, E., Koke, M., Hage, D.S.: Review: Glycation of human serum albumin. *Clin. Chim. Acta* **425**, 64–76 (2013)
11. Nagai, R., Mori, T., Yamamoto, Y., Kaji, Y., Yonei, Y.: Significance of advanced glycation end products in aging-related disease. *Anti-Aging. Med.* **7**, 112–119 (2010)
12. Han, M., Wang, X., Ding, H., Jin, M., Yu, L., Wang, J., Yu, X.: The role of *N*-glycosylation sites in the activity, stability, and expression of the recombinant elastase expressed by *Pichia pastoris*. *Enzyme. Microb. Technol.* **54**, 32–37 (2014)
13. Wautier, J.-L., Guillausseau, P.-J.: Diabetes, advanced glycation endproducts and vascular disease. *Vasc. Med.* **3**, 131–137 (1998)
14. Peppas, M., Uribarri, J., Vlassara, H.: Glucose, advanced glycation end products, and diabetes complications: what is new and what works. *Clin. Diab.* **21**, 186–187 (2003)
15. O'Connor, S.E., Imperiali, B.: Modulation of protein structure and function by asparagine-linked glycosylation. *Chem. Biol.* **3**, 803–812 (1996)
16. Vitek, M.P., Bhattacharya, K., Glendening, J.M., Stopa, E., Vlassara, H., Bucala, R., Manogue, K., Cerami, A.: Advanced glycation end products contribute to amyloidosis in Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 4766–4770 (1994)
17. Goldin, A., Beckman, J.A., Schmidt, A.M., Creager, M.A.: Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* **114**, 597–605 (2006)
18. Stivala, A., Wybrow, M., Wirth, A., Whisstock, J.C., Stuckey, P.J.: Automatic generation of protein structure cartoons with Pro-origami. *Bioinformatics* **27**, 3315–3316 (2011)
19. Beck, D.A., Daggett, V.: Methods for molecular dynamics simulations of protein folding/unfolding in solution. *Methods* **34**, 112–120 (2004)
20. Krishnan, A., Giuliani, A., Zbilut, J.P., Tomita, M.: Implications from a network-based topological analysis of ubiquitin unfolding simulations. *PLoS ONE* **3**, e2149 (2008)
21. Wormald, M.R., Dwek, R.A.: Glycoproteins: glycan presentation and protein-fold stability. *Structure* **7**, R155–160 (1999)
22. Jafari-Aghdam, J., Khajeh, K., Ranjbar, B., Nemat-Gorgani, M.: Deglycosylation of glucoamylase from *Aspergillus niger*: effects on structure, activity and stability. *Biochim. Biophys. Acta* **1750**, 61–68 (2005)
23. Sola, R.J., Griebenow, K.: Effects of glycosylation on the stability of protein pharmaceuticals. *J. Pharm. Sci.* **98**, 1223–1245 (2009)
24. Johansen, M.B., Kiemer, L., Brun, S.: Analysis and prediction of mammalian protein glycation. *Glycobiology* **16**, 844–853 (2006)
25. Watkins, N.G., Thorpe, S.R., Baynes, J.W.: Glycation of amino groups in protein. *J. Biol. Chem.* **260**, 10629–10636 (1985)
26. Sen, S., Bose, T., Roy, A., Chakraborti, A.S.: Effect of non-enzymatic glycation on esterase activities of hemoglobin and myoglobin. *Mol. Cell. Biochem.* **301**, 251–257 (2007)
27. Iannuzzi, C., Maritato, R., Irace, G., Sirangelo, I.: Glycation accelerates fibrillization of the amyloidogenic W7FW14F apomyoglobin. *PLoS ONE* **8**, e80768 (2013)
28. Roy, A., Sil, R., Chakraborti, A.S.: Non-enzymatic glycation induces structural modifications of myoglobin. *Mol. Cell. Biochem.* **338**, 105–114 (2010)
29. Ansari, N.A., Dash, D.: Amadori glycated proteins: role in production of autoantibodies in diabetes mellitus and effect of inhibitors on non-enzymatic glycation. *Aging Dis.* **4**, 50–6 (2013)
30. Cusimano, B.L., Booth, A.A., Todd, P., Hudson, B.G., Khalifah, R.G.: Unusual susceptibility of heme proteins to damage by glucose during non-enzymatic glycation. *Biophys. Chem.* **105**, 743–755 (2003)
31. Azami-Movahed, M., Shariatzi, S., Sabbaghian, M., Ghasemi, A., Ebrahim-Habibi, A., Nemat-Gorgani, M.: Heme binding site in apomyoglobin may be effectively targeted with small molecules to control aggregation. *Int. J. Biochem. Cell Biol.* **45**, 299–307 (2013)
32. Banerjee, S., Chakraborti, A.S.: Structural alterations of hemoglobin and myoglobin by glyoxal: a comparative study. *Int. J. Biol. Macromol.* **66**, 311–8 (2014)