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Deferiprone: structural and functional modulating agent of hemoglobin fructation

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Abstract Diabetic complication arises from the presence of advanced glycation end products in different sites of the body. Great attention should be paid to recognizing anti-glycation compounds. Here, deferiprone as an oral iron chelator drug administrated in treatment of β-thalassemic patients was selected to find its effect on the fructation of hemoglobin (Hb). Our results indicated that deferiprone could prevent the AGE and carbonyl formation via inhibition of structural changes in the structure of Hb during the fructation process. Moreover, deferiprone can preserve peroxidase and esterase activities of fructated Hb similar to native Hb. Therefore, deferiprone can be introduced as an anti-glycation drug to prevent the AGE formation.

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Introduction

Proteinopathies (protein conformational-related diseases) like prion encephalopathy, cystic fibrosis, type II diabetes, Alzheimer's and Huntington's diseases, result from protein aggregation and misfolding. Glycation is a condition that leads to proteinopathy by affecting the structure and function of proteins and has an essential role in diabetes [1]. These alterations depend on the concentration, nature and half life of proteins as well as sugar concentration [2]. During the glycation process, where a protein is exposed to high sugar concentrations, Schiff's base, Amadori products and advanced glycation end products (AGEs) are consecutively formed. The presence of AGE adducts on specific residues deforms the arrangement of protein structure, namely β-structure formation and protein aggregation [3]. Numerous pathogeneses arise from AGEs [4], specifically, glycation leads to loss of heme and destroys the assembly of hemoglobin (Hb) chains in diabetic patients [5].

Similar to glucose, fructose follows up the glycation process. It is known as fructation and it is considered as a highly probable event in diabetic conditions [6]. Fructose accumulates in the organs, such as the eye lens, where the sorbitol pathway is active [7]. Recent studies have indicated that fructose interacts with Hb and causes protein structural and functional alterations [8, 9]. Fructose is more reactive than glucose in glycation, AGE and carbonyl formation processes [7, 10] and has, therefore, attracted more attention as a glycating agent. On the other hand, glycated proteins have been observed in some non-diabetic pathological states such as myocardial infarction, nephrotic

syndrome and chronic renal failure [7]. Moreover, glycated Hb has been reported to increase in anemic patients despite the shortened life span of erythrocytes [11, 12].

β-Thalassemia major patients with chronic transfusion encounter iron overload disorder and free hydroxyl radicals production that damage some organs [13]. In patients with insufficient chelation therapy, endocrine glands and heart and liver malfunctions result from iron overload [14]. Deferiprone, as the orally bioavailable iron chelator, is one of the clinically approved drugs for treatment of β-thalassemia patients.

Because of its low molecular weight and high lipophilicity, deferiprone has an enhanced capacity to permeate red blood cell (RBC) membranes and can be utilized to remove pathologic deposits of chelatable iron from thalassemic and sickle RBC [15]. Deferiprone is metabolized in the liver and rapidly eliminated with a half-life of 47–143 min in healthy and 2.3 h in β-thalassemia patients. The most serious potential side effect of deferiprone in human is a transient neutropenia, which occurs in less than two percent (2 %) of patients [16].

Until now, some compounds with reactive nucleophilic groups such as aminoguanidine and pyridoxamine chelate carbonyl compounds and inhibit AGEs accumulation. In continuation of our studies on the glycation of albumin [17–21], here, we report the impact of deferiprone on structural and functional changes of beef Hb during fructation. Deferiprone as a chelator drug can potentially inhibit AGEs formation due to the nucleophilic group present in its structure. In Addition, Beef Hb was employed to study the AGE inhibition by deferiprone as a model protein and because it has different uses in biotechnology such as oxygen carrier and AGE ELISA Kit.

Materials and methods

Materials

Beef Hb, guanidine hydrochloride, mannitol, 2,4-dinitrophenylhydrazine (DNPH), trichloroacetic acid (TCA), p-nitrophenyl acetate (p-NPA), o-dianisidine, bicinchoninic acid (BCA) and hydrogen peroxide were purchased from Sigma (USA). The membrane filters (0.2 μm pore size, 25 mm in diameter) were obtained from Whatman (UK). Fructose, ethanol, ethyl acetate, citric acid, sodium citrate and sodium azide were purchased from Merck (Germany). Deferiprone was received as a gift from Arasto Pharmaceutical Chemicals Inc., Tehran, Iran. All other materials were of analytical grade. A 50 mM phosphate buffer solution at pH 7.4 (Na_2HPO_4/NaH_2PO_4) was used throughout the study, unless otherwise stated. All solutions were prepared with deionized water.

Instruments

Spectrophotometric measurements were carried out using a Shimadzu UV-3100 spectrophotometer. Spectrofluorimetric measurements were performed using a Cary Eclipse fluorescence spectrophotometer equipped with a thermostatically controlled cuvette compartment. The fluorescence of tryptophan residue in diluted samples (3 μM) was monitored with excitation at 285 nm. In addition, fluorescence spectra for the glycated samples were obtained with the excitation wavelength of 370 nm for detection of AGE.

Circular dichroism (CD) spectra were recorded on a Jasco J720 spectropolarimeter at 25 °C with a path length of 0.1 cm. The results were expressed as the molar ellipticity [θ], which is defined as $[\theta] = \theta/\{10 \times \text{number of amino acid} \times l \times C\}$, where C is the molar concentration of the sample, and l is the length of the light path in cm. Far-UV CD (200–260 nm range) measurements were used to monitor the secondary structural change of protein (3 μM). Also, helix content of the samples was calculated using CDNN CD spectra deconvolution software (Version 2.1).

Thermal stability of the samples was evaluated by differential scanning calorimetry (DSC) measurements with a Nano-DSC II differential scanning microcalorimeter (Setaram, USA) in the temperature range of 25–85 °C at a heating rate of 1 °C/min. The melting point (Tm), calorimetric denaturation enthalpy (ΔHcal) and partial molar heat capacity of the protein samples (ΔCp) were determined based on the method of Privalov and Potekhin [22].

Procedures

Hb concentration was determined spectrophotometrically with an absorption coefficient of 125 mM^{-1} cm^{-1} at 415 nm on monomer basis. Solutions of Hb (2 mM in phosphate buffer + 0.1 mM sodium azide) were incubated in capped vials under sterile conditions as the following: a control sample (Hb), a sample with fructose (50 mM) (F-Hb), a sample with fructose (50 mM) + deferiprone (250 μM, denoted as F-HB-D), and a sample with deferiprone (250 μM, denoted as Hb-D). Then, the samples were incubated at 37 °C for 5 days. The samples were then dialyzed against phosphate buffer at 4 °C for 16 h. It should be noted that all the dishes were autoclaved prior to use to inactivate proteases, and all the solutions were filter-sterilized with 0.2 μm membrane. After dialysis, the concentrations of protein samples were determined in triplicates by BCA protein assay. Then, the samples were aliquoted and stored at −20 °C until analysis.

In order to obtain information about the functional properties of the fructated Hb, the peroxidase and esterase activities of the fructated Hb samples were evaluated

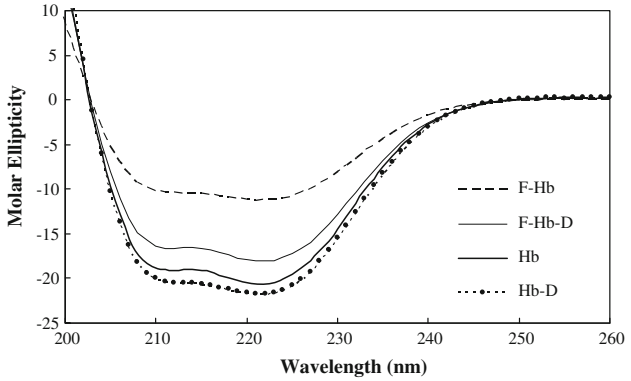

Fig. 1 Far-UV CD spectra of the control (Hb), F-Hb, F-Hb-D and Hb-D samples after 5 days incubation at 37 °C in 50 mM phosphate buffer solution (pH 7.4). Hb, fructose and deferiprone concentrations in samples are 2 mM, 50 mM and 250 μM, respectively

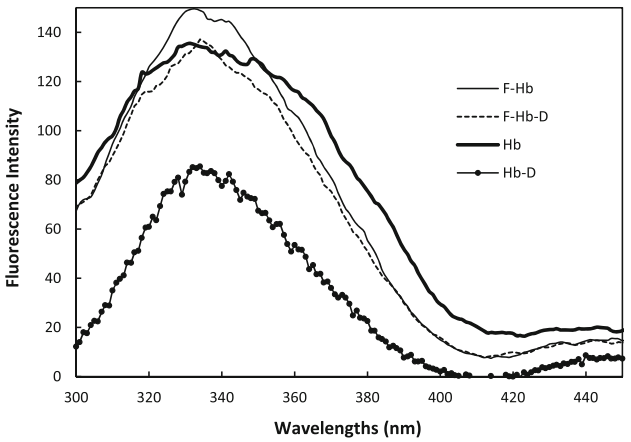

Fig. 2 Fluorescence spectra after excitation at 285 nm of the control (Hb), F-Hb, F-Hb-D and Hb-D samples after 5 days incubation at 37 °C in 50 mM phosphate buffer solution (pH 7.4). Hb, fructose and deferiprone concentrations in samples are 2 mM, 50 mM and 250 μM, respectively

according to the method proposed by Everse et al. [23] and Elbaum and Nagel [24]. Peroxidase activity was measured in a reaction mixture containing protein samples (0.5 μM) + citrate buffer, pH 5.4 (50 mM) + 0.002 % o-dianisidine. After adding hydrogen peroxide (17.6 mM) to the reaction mixture, the absorbance at 450 nm was recorded for 2 min. In order to evaluate the esterase activity, the reaction mixture containing protein samples (0.5 μM) + p-NPA (1.5 mM) was prepared and its absorbance was measured at 400 nm for 2 min.

Carbonyl stress was measured based on the method of Levine et al. [25] in a solution of protein samples (12 μM) + hydrogen peroxide (2 mM) + mannitol (25 μM) at 37 °C. Then, 50 μL DNPH (10 mM) was added and mixed. After the addition of 250 μM TCA (20 %) and centrifugation, the pellet was collected and washed three times with 1 mL ethanol:ethyl acetate (1:1) mixture. The pellet was then dissolved in 500 μL guanidine hydrochloride (6 M) and incubated for 15 min at 37 °C. Carbonyl contents were determined from the absorbance at 370 nm using a molar absorption coefficient of 22,000 M^{-1} cm^{-1}.

All of the experiments were done three times independently and the results were expressed as mean value ± standard deviation (SD). Statistical analyses were performed using GraphPad software. A p value of $p < 0.05$ was considered as statistically significant.

Results

Figure 1 shows far-UV CD spectra of the control, F-Hb, F-Hb-D and F-D samples in the range of 200–260 nm, denoting an alteration in the secondary structure of Hb. The far-UV CD spectrum of Hb is characterized by the presence of two strong negative bands at 208 and 222 nm, related to the helical character of the protein. Upon incubation of Hb with fructose, there was a loss of helical structure in the F-Hb sample deducing from the decreases in the negative ellipticities at 208 and 222 nm, compared to the control sample. On the other hand, the far-UV CD spectrum of F-Hb-D sample showed an increase in the negative ellipticity, compared to the F-Hb sample. This is a direct evidence for inhibitory effect of this drug on the fructation process. Helix content of Hb, F-Hb, F-Hb-D and Hb-D samples were 35.9, 24.2, 30.7 and 41.7 %, respectively.

Figure 2 shows the intrinsic fluorescence spectra of the control, F-Hb, F-Hb-D and Hb-D samples. In the spectrum of F-Hb sample, the fluorescence intensity of the emission peak at 340 nm (related to Trp residue) increased, indicating the occurrence of changes in the protein structure resulting from the reaction with fructose. However, the fluorescence intensity at 340 nm for the Hb-D sample decreased as compared to the control sample. The fluorescence intensity at 340 nm for Hb-F-D sample is similar to that of the control.

Figure 3 represents the temperature dependence of the molar heat capacity (Cp) of the control, F-Hb and F-Hb-D samples. Based on these results, melting temperatures (Tm) of 68.5, 68.5 and 69.1 °C were obtained for the control, F-Hb-D and F-Hb samples, respectively. The values of ΔHcal, ΔCp and Tm for the control, F-Hb-D and F-Hb samples are shown in Table 1. The results showed that the F-Hb sample has a higher Tm, ΔHcal, ΔCp compared to the control sample. The results showed that Tm and ΔCp in F-Hb-D and control are similar. Enthalpy in F-Hb-D sample reduced as compared to the control due to alteration in intermolecular interactions.

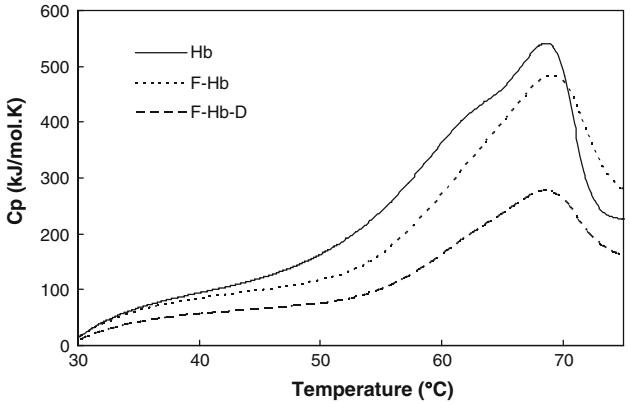

Fig. 3 DSC thermogram of the control (Hb), F-Hb and F-Hb-D samples after 5 days incubation at 37 °C in 50 mM phosphate buffer solution (pH 7.4). Hb, fructose and deferiprone concentrations in samples are 2 mM, 50 mM and 250 μM, respectively

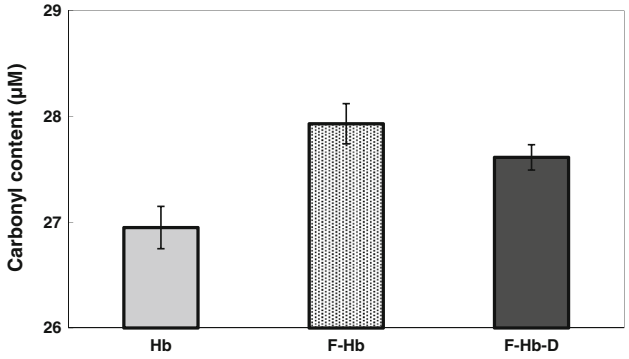

Fig. 5 Carbonyl content of the control, F-Hb and F-Hb-D samples after 5 days incubation at 37 °C in 50 mM phosphate buffer solution (pH 7.4). Hb, fructose and deferiprone concentrations in samples are 2 mM, 50 mM and 250 μM, respectively. Results are expressed as mean value of three independent experiments ±SD. Results are very statistically significant ($p < 0.04$)

Table 1 ΔHcal and ΔCp for the control, F-Hb and F-Hb-D samples

	ΔHcal (kJ/mol)	ΔCp (kJ/mol K)
Control	40.21 ± 0.27	79.1 ± 1.9
F-Hb	26.33 ± 0.34	147.5 ± 7.5
F-Hb-D	13.78 ± 0.06	74.7 ± 3.5

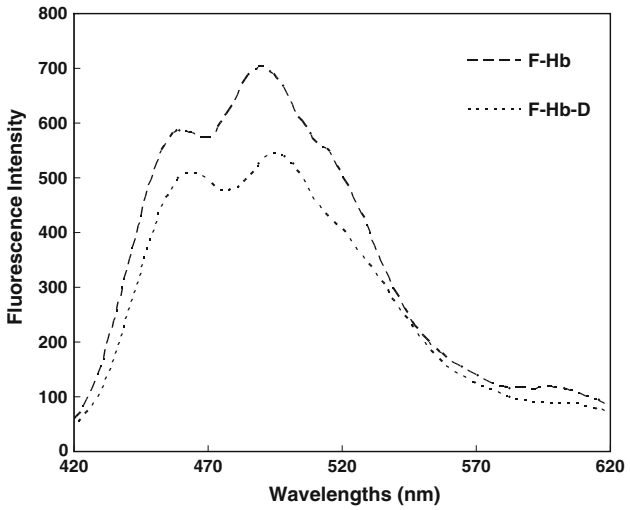

Fig. 4 Fluorescence spectra after excitation at 370 nm of the F-Hb and F-Hb-D samples after 5 days incubation at 37 °C in 50 mM phosphate buffer solution (pH 7.4). Hb, fructose and deferiprone concentrations in samples are 2 mM, 50 mM and 250 μM, respectively

Figure 4 shows the fluorescence spectra of AGEs. In this Figure, the temporal accumulation of fluorescence AGEs is shown after excitation at 370 nm and measurement of the emission at 420–620 nm. The emission values indicate that accumulation of AGE in the F-Hb sample is higher than the F-Hb-D one.

Based on the results presented in Figs. 2 and 4, there was a simultaneous decrease in the Trp emission intensity and the emission intensity between 420 and 620 nm for the F-Hb-D sample. This confirms a positive effect of deferiprone to prevent the fructation reaction and AGE formation.

In order to further inspect the drug effect on the fructation of Hb, an index of protein oxidation and oxidative stress was measured using the measurement of carbonyl formation. The results are presented in Fig. 5. In the experiment, mannitol was used as an inhibitor for carbonyl formation [25]. The results indicated that mannitol was more effective in preventing carbonyl formation in the control sample, rather than F-Hb. In addition, the carbonyl content of the F-Hb sample was higher than that of the control sample and deferiprone decreased the carbonyl content of F-Hb sample.

Heme proteins represent peroxidase and esterase activities. Previous studies [[26–28] g] have shown that glycation reduces the peroxidase activity and increases the esterase activity of Hb. Also, the esterase activity progressively increases with the glucose level in diabetes. As for the peroxidase activity, Hb interacts with H_2O_2 to afford ferrylhemoglobin. Ferrylhemoglobin is a very potent oxidant and oxidizes electron donor compounds [28]. Enzyme-like activities of different Hb samples were analyzed and the results are shown in Fig. 6. F-Hb sample exhibited less peroxidase activity than the control sample, while deferiprone improved the peroxidase activity of Hb. In addition, the esterase activity of Hb was analyzed using p-NPA as the substrate. Figure 6 shows that F-Hb sample

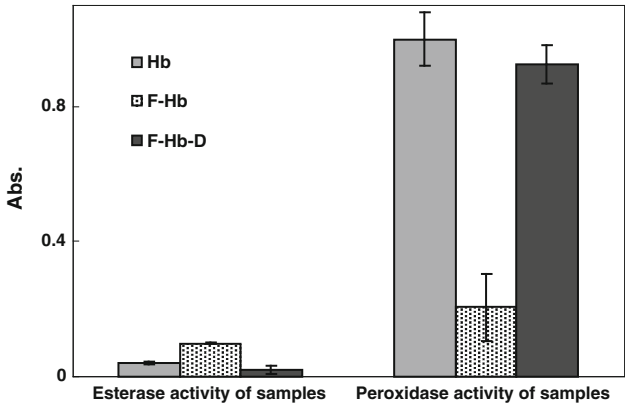

Fig. 6 Peroxidase and esterase activity of the control (Hb), F-Hb and F-Hb-D samples after 5 days incubation at 37 °C in 50 mM phosphate buffer solution (pH 7.4). Hb, fructose and deferiprone concentrations in samples are 2 mM, 50 mM and 250 μM, respectively. Results are expressed as mean value of three independent experiments ±SD. Results are very statistically significant ($p < 0.001$ for peroxidase activity and $p < 0.005$ for esterase activity)

had more esterase activity than the control sample, and deferiprone decreased the esterase activity of F-Hb sample.

Discussions

Compounds that can directly inhibit protein glycation might be useful for preventing the diabetic complications. In this study, structural and functional studies have been employed to study the effect of deferiprone, as an oral iron chelator drug, on the fructation of Hb. Deferiprone concentration used in this study (250 μM) was well within the limit of the therapeutic relevance [15].

From far-UV CD spectrum, it can be concluded that fructation of Hb following five-days of incubation induces loss of helical structure of Hb. However, deferiprone induces helicity (increase the negative ellipticity) of Hb. Therefore, deferiprone can modulate the fructation effects and compensates the helical loss in Hb. These effects result from the deferiprone-protein interaction [15].

Our results showed that deferiprone leads to a decrease in the Trp fluorescence resulting from fructation. This reveals the Hb-drug interaction followed by modulation of fructation effects.

Regarding the AGE formation based on the values of the fluorescence intensities between 420 and 620 nm, production of fluorescence AGEs in fructated Hb decreased in the presence of deferiprone. Also, according to the DSC data, thermostable nature of fructated Hb was changed in the presence of deferiprone.

Based on the results of the enzymatic assays, the esterase activity of Hb increased during the fructation and its enhancement was hindered by deferiprone. It is well-known that the esterase activity of Hb increased during fructation of Hb due to conformational changes in the protein structure [27]. These conformational changes cause histidine β2 residue to be better juxtaposed and to exert its hydrolytic activity on the p-NPA substrate [27]. It should be mentioned that lysine and arginine residues of the protein have prone to modify by glycation. Based on previous docking study [28], the probable sites of tetrameric oxyhemoglobin modification by fructose are Lys7α, Lys 127α and Lys 66β that are close to His β2. However, the interaction of deferiprone with Hb reduces he conformational changes and esterase activity of Hb. This confirms the maintenance of structural integrity of Hb with deferiprone.

The results of the peroxidase activity assay of F-Hb against the o-dianisidine substrate showed that this activity was lowered upon fructation. It is reasonable to assume that the conformational changes and heme loss due to fructation of Hb reduce the entry of the substrate molecule to the heme pocket, leading to a decrease in the peroxidase activity of the protein [28]. Also, fructation of Hb modifies Lys 66β residue charge near the heme group, results in the weakening of the heme-globin linkages and leads to iron release [28]. On the other hand, electrostatic interaction of deferiprone with F-Hb [29] may modify the heme pocket, results in enhanced accessibility of the o-dianisidine substrate and promotion of peroxidase activity.

Carbonyl stress is one of the diabetic complications which has resulted in glycation of proteins [29]. In this reaction, iron of Hb reacts with H_2O_2, affords radicals, oxidizes amino acid residues, and finally induces carbonyl group formation in proteins. Deferiprone can also inhibit the carbonyl formation by removing iron [29]. It should be noted that the carbonyl content in F-Hb is higher than Hb because H_2O_2 releases more iron from F-Hb than from Hb.

An ESR spectroscopic study has demonstrated a decreased mobility for the lysine residue in the glycated Hb due to a change in the protein conformation [30]. Fluorescence measurements also revealed that there is a significant interaction between deferiprone and Trp residues of Hb; this may be indicative of the proximity of Trp to the binding site of deferiprone [15]. Therefore, deferiprone binds to Hb with high affinity [15] and reinforces the protein structure against any distortion and conformational changes resulting from fructation. Moreover, deferiprone can stabilize the protein from electrostatic point of view via hydrogen bond like electrostatic attractions [15]; the drug can modify the electrostatic character of hemoglobin (losses in the positive charge). On the other hand, the compounds bearing the reactive nucleophilic sites can trap the carbonyl groups present in the fructated Hb structure and inhibit the AGE formation [31]. Deferiprone with two

nucleophilic groups can also inhibit the AGE formation via this mechanism.

Beside the direct interaction of deferiprone-Hb leading to "latching" the protein structure from distortion, the drug can have positive effects from another point of view. It has been reported that metallic ions can promote the glycation reaction [30]. Oxidation reactions via metallic ion lead to formation more permanent, irreversible protein glycation products, such as the glycoxidation products [32]. Deferiprone chelates metal ions such as iron, aluminum and some other heavy metal ions [17, 33–35] and also chelates carbonyl groups. Therefore, deferiprone can inhibit the glycation process in beef Hb in vitro.

Conclusions

In summary, our results showed that deferiprone inhibits the fructation of beef Hb and complications such as AGE formation. These results were obtained by comparing the structural and functional changes of beef Hb during in vitro fructation. The drug causes the protein to retain its structure and functions under fructation conditions.

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References

1. Hayden M, Tyagi SC, Kerklo MM, Nicolls MR (2005) Type 2 diabetes mellitus as a conformational disease. J Pancreas 6:287–302
2. Brownlee M (2000) Negative consequences of glycation. Metabolism 49:9–13
3. GhoshMoulick R, Bhattacharya J, Roy S, Basak S, Dasgupta AKr (2007) Compensatory secondary structure alterations in protein glycation. Biochim Biophys Acta 1774:233–242
4. Lapolla A, Traldi P, Fedele D (2005) Importance of measuring products of non-enzymatic glycation of proteins. Clin Biochem 38:103–115
5. Thomas MC, Tsalamandris C, Macisaac R, Jerums G (2006) Functional erythropoietin deficiency in patients with Type 2 diabetes and anaemia. Diabet Med 23:502–509
6. Schalkwijk GC, Stehouwer CDA, Hinsberg VWM (2004) Fructose-mediated non-enzymatic glycation: sweet coupling or bad modification. Diabetes/Metab Res Rev 20:369–382
7. Chowdhury TA, Lasker SS (1998) Elevated glycated hemoglobin in nondiabetic patients is associated with an increased mortality in myocardial infarction. Postgrad Med J 74:480–481
8. Bakhti M, Habibi-Rezaei M, Moosavi-Movahhedi AA, Khazaei MR (2007) Consequential alterations in haemoglobin structure upon glycation with fructose: prevention by acetylsalicylic acid. J Biochem 141:827–833
9. Bhattacherjee A, Chakraborti AS (2011) Fructose-induced modifications of myoglobin: change of structure from met (Fe3+) to oxy (Fe2+) form. Inter J Biol Macromol 48:202–209
10. Bunn HF, Higgins PJ (1981) Reaction of monosacharides with proteins: possible evolutionary significance. Science 213: 222–224
11. Cohen E, Ozdogan M, Timuragaoglu A (2004) Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. Acta Haematol 112:126–128
12. Sundaram RC, Selvaraj N, Vijayan G, Bobby Z, Hamide A, Rattina Dasse N (2007) Increased plasma malondialdehyde and fructosamine in iron deficiency anemia: effect of treatment. Biomed Pharmacother 61:682–685
13. Hershko C, Konijn AM, Link G (1998) Iron chelators for thalassaemia. Br J Haematol 101:399–406
14. Oliveri NF (2006) The β-thalassemias. N Engl J Med 341:99–109
15. Chakraborty D, Bhattacharyya M (2000) Deferiprone (L1) induced conformation change of hemoglobin: a fluorescence and CD spectroscopic study. Mol Cell Biochem 204:17–20
16. Kontoghiorghes GJ, Pattichi K, Hadjigavriel M, Kolnagou A (2000) Transfusional iron overload and chelation therapy with deferoxamine and deferiprone (L1). Transfus Sci 23(3):211–223
17. Sattarahmady N, Moosavi-Movahedi AA, Habibi-Rezaei M (2011) A biophysical comparison of human serum albumin to be glycated in vivo and in vitro. J Med Biochem 30:5–10
18. Sattarahmady N, Moosavi-Movahedi AA, Ahmad F, Hakimelahi GH, Habibi-Rezaei M, Saboury AA, Sheibani N (2007) Formation of the molten globule-like state during prolonged glycation of human serum albumin. Biochim Biophys Acta 1770:933–942
19. Sattarahmady N, Moosavi-Movahedi AA, Habibi-Rezaei M, Ahmadian S, Saboury AA, Heli H, Sheibani N (2008) Detergency effect of nanofibrillar amyloid for mation on glycation of human serum albumin. Carbohydrate Res 343:2229–2234
20. Sattarahmady N, Khodagholi F, Moosavi-Movahedi AA, Heli H, Hakimelahi GH (2007) Alginate as antiglycating agent for human serum albumin. Int J Biol Macromol 41:180–184
21. Sharifi E, Sattarahmady N, Habibi-Rezaei M, Farhadi M, Sheibani N, Moosavi-Movahedi AA (2009) Inhibitory effects beta-cyclodextrin and trehalose on nanofibril and AGE formation during glycation of human serum albumin. Protein Pept Lett 16(6):653–659
22. Privalov PL, Potekhin SA (1986) Scanning microcalorimetry in studying temperature-induced changes in proteins. Methods Enzymol 131:4–51
23. Everse J, Johnson MC, Marini MA (1994) Peroxidative activities of hemoglobin and hemoglobin derivatives. Methods Enzymol 231:547–561
24. Elbaum D, Nagel RL (1981) Esterase activity of hemoglobin: difference between HbA and HbS. J Biol Chem 256:2280–2283
25. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A, Ahn B, Shaltiel S, Stadtman ER (1991) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 208:464–478
26. Moulick RG, Bhattacharya J, Roy S, Basak S, Dasgupta AK (2007) Compensatory secondary structure alterations in protein glycation. Biochim Biophys Acta 1774:233–242
27. Sen S, Bose T, Roy A, Chakraborti AS (2007) Effect of non-enzymatic glycation on esterase activities of hemoglobin and myoglobin. Mol Cell Biochem 301:251–257
28. Bose T, Chakraborti AS (2008) Fructose-induced structural and functional modifications of hemoglobin: implication for oxidative stress in diabetes mellitus. Biochim Biophys Acta 1780:800–880
29. Moosavi-Movahedi AA, Mousavy SJ, Divsalar A, Babaahmadi A, Karimian K, Shafiee A, Kamarie M, Poursasan N, Farzami B, Riazi GH, Hakimelahi GH, Tsai FY, Ahmad F, Amani M, Saboury AA (2009) The effects of deferiprone and deferasirox on the structure and function of b-thalassemia hemoglobin. J Biomol Struct Dyn 27:319–329
30. Sen S, Kar M, Roy A, Chakraborti AS (2005) Effect of nonenzymatic glycation on functional and structural properties of hemoglobin. Biophys Chem 113:289–298

31. Nagai R, Murray DB, Metz TO, Baynes JW (2012) Chelation: a fundamental mechanism of action of AGE inhibitors, AGE breakers, and other inhibitors of diabetes complications. Diabetes 61:549–559
32. Fu MX, Wells-Knecht KJ, Blackledge JA, Lyons TJ, Thorpe SR, Baynes JW (1994) Glycation, glycoxidation, and cross-linking of collagen by glucose: kinetics, mechanisms, and inhibition of late stages of the maillard reaction. Diabetes 43:676–683
33. Di J, Zhang F, Zhang M, Bi S (2004) Indirect voltammetric determination of aluminum in environmental and biological samples in the presence of the aluminum chelating drugs. Electroanalysis 16:644–649
34. Fukuda S (2005) Chelating agents used for plutonium and uranium removal in radiation emergency medicine. Curr Med Chem 12:2765–2770
35. Pashalidis I, Kontoghiorghes GJ (1999) Effective complex formation in the interaction of 1,2-dimethyl-3-hydroxypyrid-4-one (Deferiprone or L1) with uranium(VI). J Radioanal Nucl Chem 242:181–184