Critical concentration of Glucose changes human serum albumin conformation: Circular Dichroism (CD)and UV Spectroscopyapproaches

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ABSTRACT

HSA plays an important role in transporting metabolites and drugs throughout the vascular system. In as much as its performance is very vital in the presents of different kinds of ligands at the specific body temperatures, its examination is crucial. This molecule can undergo increased glycation in diabetes. Therefore, glucose as the one of the most fundamental ligands dealing with albumin in human body is examined in this study at 100 mg/dl concentration in correspond to normal condition on human body, 175 mg/dl as a kidney glucose tolerance point and also 400 mg/dl as the critical point at the two most important temperatures in diabetic patients. Thermal conformational changes of (HSA) are important. These conformational alterations are accompanied by a mild alteration of secondary structures. For this reason, possible secondarystructural changes of HSA in presence of glucose has beeninvestigated by circular dichroism (CD) using Hepes bufferat the normal temperature 37˚C and 42˚C as a high fever condition.UV spectroscopystudies confirmed CD findings and indicate that critical concentration of glucoselead to generation of new structural feature of albumin similar to 42^{o}C. However, as the temperature increases from 37˚C to 42˚C this process is no more capable of responding to glucose concentration changes.These results indicate that the native form of HSA is changed in the severe diabetic condition; likewise, same consequences can be achieved as the temperature arises from 37˚C to 42˚C.

Keywords: Human serum albumin (HSA);Temperature;Structural changes; Glucose; Circular dichroism(CD); UV spectroscopy

INTRODUCTION

Human serum albumin (HSA) as a monomeric multidomainglobular protein is the most common circulating protein in blood. This molecule consistsof 585 amino acids with an average molecular weight of66500 Da[1]. This molecule representsthe major controllingelement of fluid dispersal among body compartments,and the most vital factor in regulatingplasma osmotic pressure. Furthermore,ligand binding capabilityenabled this vital molecule for carrying many endogenous and exogenous compounds. In fact, albumininfluences pharmacokinetics of many drugs, signifies the chief carrier for fatty acids, control the metabolic change of some ligands, transforms possible toxins mild, makes up for most of the anti-oxidant capacity of human plasma,and shows (pseudo-)enzymatic activities [2-4]. Additionally, albumin is a biological marker in a number of clinical disorders, adaptation mechanisms may be involved[5]. Remarkably,a number of factors are identified to influence HSA structure and dynamics, like pH, temperature, and binding of different ligands[6]. It has beenmarked that not onlyprotein activity, unfolding, and degradation but also cell functioning is highly related to protein glycation. This globular heart-shaped molecule contains three homologous domains typically specified as I (1–195), II (196–383), and III (384–585). Thesedomains are comparable bothin the amino acid sequence and in the secondary and tertiary structures.Amazingly, even in thepresence of a wide variety of ligandsalbumin conformation is completelypreserved[7-9].From recent studies, glycated albumin has biological impact on cell physiology and functioning; it is known as a marker of diabetes [3, 10, 11]. For instance,a risk factor for diabetes mellitus (DM) is urinary albumin excretion, which is independent of primary metabolic profile and increase of insulin resistance[12, 13].Moreover,

glycation has key implications for albumin performance and influence on cell.The incubation of HSA with glucose results in its non-enzymatic glycoxidation in a concentration, incubation time, and temperature dependent manner. Non-enzymatic glycation of HSA alters its conformation and function[14]. In this study, it is proposed that glucose binds to HSA and alters the structural aspects. Circular dichroism (CD) and UV spectroscopic methods,consequently,have been appliedto evaluatethe structural modifications of human serum albumin in the temperature of 37-42˚C in the presence and absence of glucose.

MATERIALS AND METHODS

Materials

Human serum albumin was purchased from Sigma chemicalCo., USA, The other substances ofreagent grade were obtained from Merck chemicalCo., Germany; also, the buffer used all through thestudy wasHepes 100 mM, pH 7.

Methods

CD spectra were recorded by a Jasco J-715 spectropolarimeter (Japan). Results are expressed as ellipticity, [θ] (degree cm2 $dmol^{-1}$), based on a mean amino acid residue weight (MRW). This value was assumed to be 113 Da for HSA. The molar ellipticity was determined as

$[\theta]\lambda = (\theta \times 100\ MRW/cl)$

Where c is the protein concentration in mM, l is the length of light path in cm and θ is the measured ellipticity in degree at a given wavelength. The data were smoothed using the Jasco J-715 software, which includes a fast Fourier-transform noise reduction routine. All experiments were repeated three times. The concentration of the protein solution was 0.5mg/ml. Percentage of secondary structures was calculated with the method of Chen et al.[15]. The UV-V absorption spectra of HSA were obtained by Unico spectrophotometer[16]. HSA incubated 5 minutes in Hepes buffer 100mM, pH 7 under the applied conditions and then the spectra were taken.The results were presented as mean±standard deviation for continuous variables.

RESULT

For examining albumin (HSA) exposure to glucose, CD technique was applied for detection of structural changes of this protein. For understanding the role of glucose on albumin structure at fever condition the mentioned experiment was repeated at 42˚C (see figure 2). Absorption UV spectroscopy is a method that is applied widely in protein studies [17-20]. Based on this method it has been reported that Acetaminophen as a pain killer induces conformational change in human serum albumin [20, 21](see figure 3). For more investigation UV spectra of albumin in the presence of two concentrations of glucose in the range of temperatures are provided, and represented in figures 4 and 5.

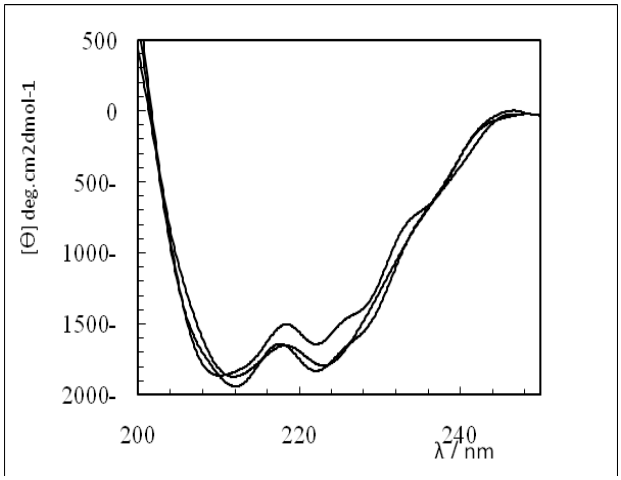

Figure 1. CD spectra of HSA in the presence of 100 mg/dl,175 mg/dl, and also 400 mg/dl concentrationsof glucose in Hepesbuffer 100 mM, pH 7 at 37˚C. Glucose concentration decreases from up to down.

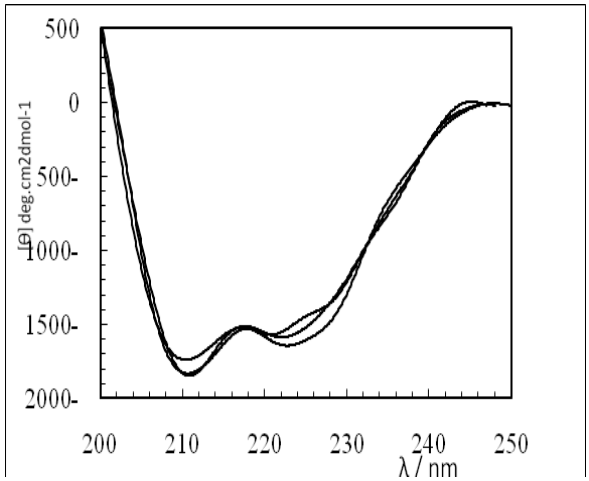

Figure 2. CD spectra of HSA in the presence of 100 mg/dl,175 mg/dl, and also 400 mg/dl concentrationsof glucose in Hepesbuffer 100 mM, pH 7 at 42˚C. Glucose concentration decreases from up to down.

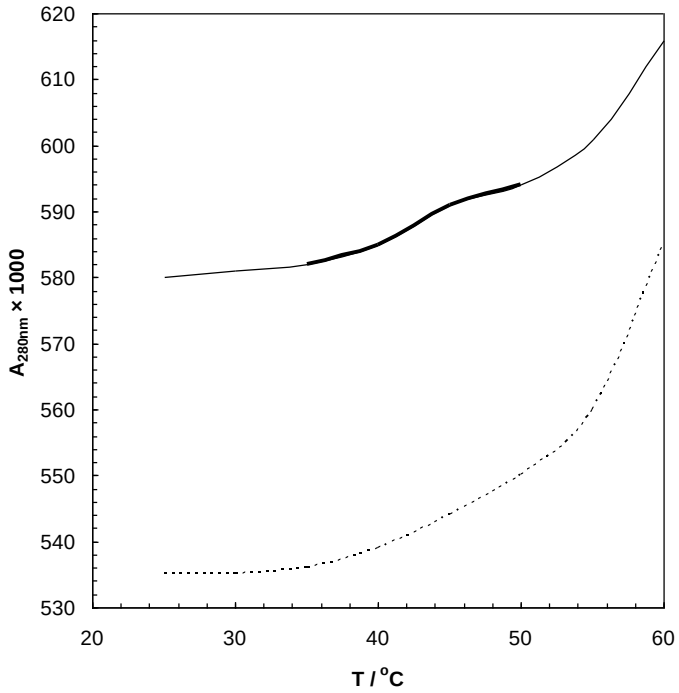

Figure 3. Acetaminophen induces conformational change in albumin, so the solid part of albumin absorption curve (up curve) is not seen in the presence of drug (low curve) [20].

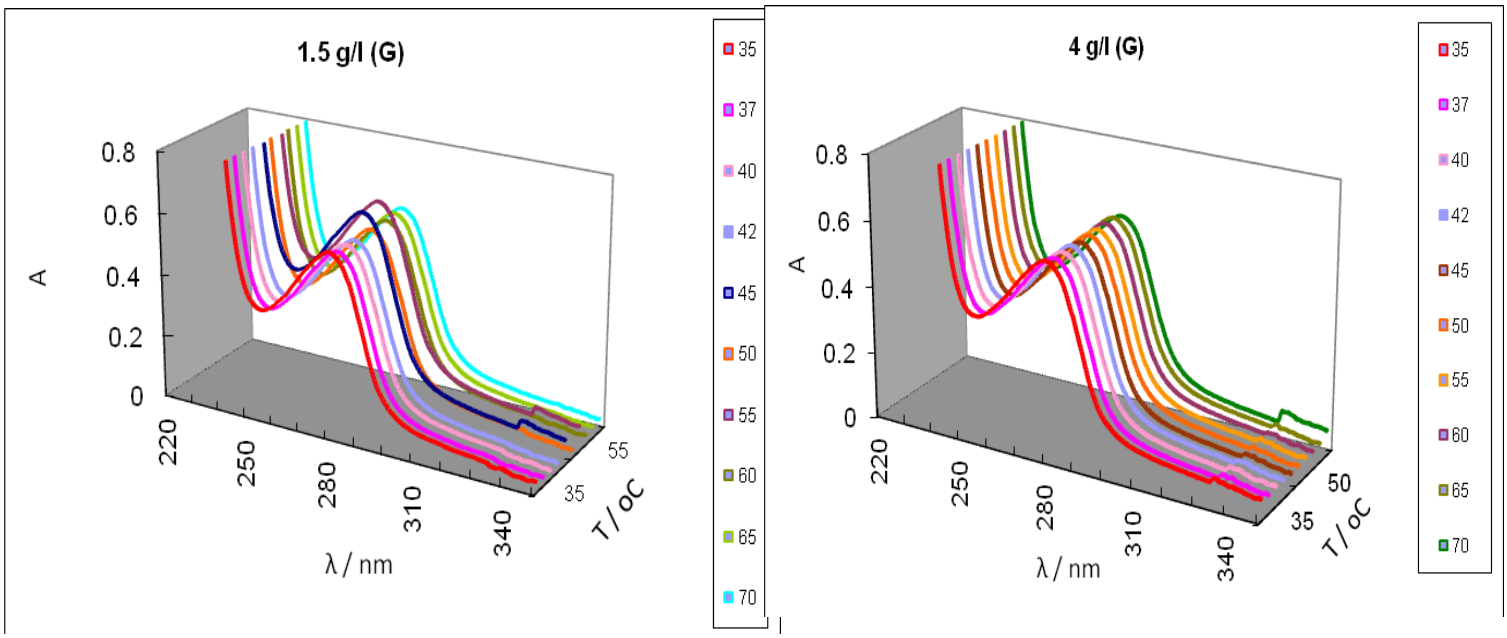

Figure 4. UV spectra of human serum albumin in the presence of 150 mg/dl concentration of glucose in the range of temperatures, Hepes buffer 100 mM and pH 7.

Figure 5. UV spectra of human serum albumin in the presence of 400 mg/dl concentration of glucose in the range of temperatures, Hepes buffer 100 mM and pH 7.

DISCUSSION

HSAas high abundanceprotein in our blood has particular tasks[22-24].HSA has remarkable ligand binding despite being monomeric; in fact, endogenous and exogenous HSA ligand binding modulation is includedby ligand-ligand competition for the same site as well as by intermolecular communication(s) within multiple clefts. HSA, furthermore, signifiesa prominentbiomarker for many diseases such ascancer, ischemia, severe acute graft-versus-host disease, and sicknessesthat require monitoring glycemic control. Here secondary structural changes associated with incubatedHSA with glucose in the 100, 175 and 400 mg/dl range in both 37˚C and 42˚Chas been studied. Near UV-CD is applied widely for the study of tertiary structural changes of protein while far UV-CD is one of the best ways for studying the secondary structural changes of proteins during phase transition [25, 26]. Accordingly, far UV-CD technique was employed for the determination of the effect of glucose on the secondary structure of HSA[27]. It can be suggest that, glucose at high concentration which is the exact dosage that diabetic patients dealing with, shows a highly modification in secondary structure of albumin which is basically related to β sheets and α helix of this molecule. Our calculation shows that albumin loses about 4% α-helix component in the presence of 400 mg/dl concentration of glucose at 37˚C. It is reported that secondary structural change may be accompanied by alteration of molecular function[1, 28], so it is an important process that glucose induces in albumin structure. The100 mg/dl as normal dose of glucose in human body and control, 175 mg/dl concentration of glucose as a glucose tolerance point for kidney and also 400 mg/dl as the critical point for glucose in diabetic conditionare selected. As it is depicted in the figure 2 this structural transition is not seen at 42˚C temperature. The 42˚C temperature corresponds to sever condition of fever in human body. Previous study [6] showed that 42˚C changes conformational structure of albumin in a reversible manor.In both cases conformational changes are accompanied by mild alteration of α-helix component of albumin.

As it is shown in figure 3[20], UV spectroscopy method is a suitable technique for analysis of protein structural change in some cases, on the other hand CD findings confirmed relationship between glucose concentrations, temperature and Albumin conformational change; here UV spectra (see figures 4 and 5) are taken and compared with CD results. UV findingsindicate that albumin structure in the presence of sub threshold dosage of glucose has a flexible unit and responses to temperature alteration in pre-denaturation range, but arising glucose concentration to 400 mg/dl diminishes this ability of albumin. This finding verifies CD findings. To sum up, it is suggested that thermal conformational changes may be alike to conformational changes of albumin in the presence of glucose at 37^{o}C.

CONCLUSION

It can be concluded that, high doses of glucose such as diabetic conditioncan possibly effect on albumin structure and function.It seems thatthis effect of glucose is similar to the effect of fever on structure and function of albumin.

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