Effect of carbonyl compounds on red blood cells deformability

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Abstract

The effect of Maillard reaction on red blood cells (RBC) deformability was investigated. Exposure of RBC to carbonyl compounds (DL-glyceraldehyde, glyoxal, glycolaldehyde, 3-deoxyglucosone, and D-glucose) leading to Maillard reaction caused a marked decrease in RBC deformability even at 1 mM level. The decrease rate depended on the kind of carbonyl compounds, in which both DL-glyceraldehyde and glyoxal significantly decreased the RBC deformability (p < 0.05). In addition, the decrease rate also differed among volunteers tested, indicating that the sensitivity against carbonyl compounds varies among them. In order to elucidate the mechanism of the decrease in RBC deformability, RBC was exposed to carbonyl compounds in the presence of aminoguanidine which is the inhibitor of AGE formation in Maillard reactions. Aminoguanidine inhibited the decrease in RBC deformability by DL-glyceraldehyde and glyoxal. When Hb which has a high reactivity with carbonyl compounds was incubated with those carbonyl compounds, DL-glyceraldehyde and glyoxal showed the high reactivity with Hb compared with other carbonyl compounds. These results indicate that Maillard reaction between RBC proteins and carbonyl compounds leads to the decrease in RBC deformability. On the other hand, O2•− generated by carbonyl compounds involved in lowering the deformability only to a negligible level.

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Keywords: Red blood cell; Deformability; Carbonyl compounds; Maillard reaction; Glyceraldehydes; Glyoxal; Aminoguanidine; Hemoglobin; Superoxide anion; Diabetes

Elevated blood glucose is an important and eventually modifiable cardiovascular risk factor, responsible for the several-fold increase in cardiovascular mortality of patients with diabetes mellitus [1]. Morbidity and mortality change proportionally with the degree of glycemic control [2,3]. The mechanism by which hyperglycemia affects both the macro- and microcirculation is still not well understood. One of the mechanisms may be an impairment of blood rheology, hereby increasing the mechanical stress and leading to structural alterations of the vessel wall. Patients with diabetes mellitus may have hemorrhheological abnormalities, e.g., increased blood viscosity [4–7] and decreased red blood cells (RBC) deformability [8] have been described. These issues are, however, controversial, as others have found normal blood viscosity [9], normal RBC deformability [4,10,11], and a normal RBC lifespan [12].

Although an elevated level of glucose has been thought to play a primary role via the Maillard reaction in increased glycation and crosslinking in diabetic tissues [13], the nonenzymatic glycation is also known to result from the action of various metabolites and the intermediates of the Maillard reaction other than glucose. Especially, glyoxal and glycolaldehyde have been recently reported to contribute to Nε-carboxymethyllysine (CML) formation, one of the advanced glycosylation end products (AGEs), and protein crosslinking under...
physiological conditions [14]. Moreover, multiple intermediates in the glycolytic and polyol pathways were capable of nonenzymatically modifying proteins and, among them, dihydroxyacetone phosphate, glyceraldehyde, and glyceraldehyde-3-phosphate were highly reactive agents that in the micromolar range of concentrations formed more AGEs much faster than 20mM glucose [15].

As already mentioned above, it is shown that patients with diabetes mellitus may have decreased RBC deformability. Since several carbonyl compounds are present at high concentration in the blood of patients with diabetes mellitus, it is conceivable that the Maillard reaction between RBC proteins and those carbonyl compounds could lead to decrease RBC deformability. In the present investigation, we examined the influence of carbonyl compounds contributing to Maillard reaction on RBC deformability and tried to elucidate the mechanism of the decrease in RBC deformability.

Materials and methods

Reagents. The following chemicals were obtained from Sigma Chemical (St. Louis, MO, USA): d,L-glyceraldehyde, glycoaldehyde (dimer), n-glucose, 2,3-diaminonaphthalene, hemoglobin (Hb) from bovine erythrocytes, 4,5-dihydroxy-1,3-benzene disulfonic acid (Tirion), and aminoguanidine hydrochloride. Xanthine oxidase (EC 1.2.3.2; 0.29U/mg) from buttermilk was purchased from Oriental Yeast (Tokyo, Japan). D-Deoxyglucosone and 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate sodium salt (WST-1) were obtained from Dojindo Laboratories (Kumamoto, Japan). 5.5-Dimethyl-1-pyrroline-N-oxide (DMPO) was obtained from Labotec (Tokyo, Japan). Glyoxal (40% aqueous solution) and 2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-one hydrochloride (MCLA) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Hypoxanthine (HPX) and 3-methyl-2-benzothiazolone hydrazono hydrochloride (MBTH) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Nacalai Tesque (Tokyo, Japan), respectively. The F-kit for D-glucose was obtained from Roche (Roche, Germany). The other chemicals were of the highest grade available and were used without further purification. All solutions used on RBC deformability measurement were prepared with Heps-buffered NaCl solution (HBS; 141mM NaCl, 10mM Heps-Na buffer, and 287mOsm/kg H2O, pH 7.4).

Preparation of RBC. Venous blood from the antecubital vein of healthy male and female adults was collected into a disposable syringe with a 21 gauge needle using 1/10 volume of 3.3% trisodium citrate as an anticoagulant. After centrifugation at 1300g for 10min, the plasma was washed three times by repeating the resuspension with HBS and centrifugation at 800g, 600g, and 500g, respectively.

Exposure of carbonyl compounds to RBC. The RBC suspension was prepared with HBS, the hematocrit (Hct) value adjusted to an adequate value for experiments. After the RBC suspension was precubated at 37°C for 10min in a water bath with gentle shaking, 1mM carbonyl compound was added. The mixture was incubated at 37°C for 1h.

Exposure of carbonyl compounds to RBC in the presence of aminoguanidine. After 10mM aminoguanidine was added to the RBC suspension, the mixture was precubated at 37°C for 10min. After addition of 1mM dl-glyceraldehyde or glyoxal, the mixture was incubated at 37°C for 1h.

Exposure of O2 to RBC. The RBC suspension was precubated at 37°C for 10min. After the addition of 0.1mM hypoxanthine and xanthine oxidase, the mixture was incubated at 37°C for 1h.

RBC deformability. RBC deformability was estimated in terms of RBC deformability through the nickel mesh filter of the RBC suspension. The Hct value of the RBC suspension was adjusted to be 3%. The filtration through the nickel mesh was performed by means of the vertical-tube method [16,17]. The filterability of the RBC was evaluated from pressure (P)-flow rate (Q) relationship of the RBC suspension. The nickel mesh (Dainippon Printing, Tokyo, Japan) is a new porous thin metal film produced to our specification using a photofabrication technique. We specified that the nickel mesh should have a diameter of 13mm, a thickness of 11µm, a pore diameter of 4.59µm, an interpore distance of 35µm, and an effective filtration area of 0.50cm². Prior to the nickel mesh filtration experiment, the suspending medium was filtered through a Millipore filter with a pore size of 0.22µm to avoid otherwise inevitable microdust contamination. Since one set of the nickel mesh was reused for each experiment after an ultrasonic washing, all experimental data were obtained employing the same mesh. Filtration experiments were carried out at room temperature (26 ± 1°C).

Incubation of Hb with carbonyl compounds. The reaction mixture contained 10mg/ml Hb and 1mM carbonyl compounds. After incubating at 37°C for 1h, Hb was removed by filtration with ultrafiltration membrane (<10,000) (Kurashiki Bouseki, Osaka, Japan).

Determination of d,L-glyceraldehyde. The concentration of dl-glyceraldehyde was determined by GC (Shimadzu gas chromatograph GC-14A equipped with a flame ionization detector) analysis by derivatization using trimethylsilylate as described previously [18].

Determination of glyoxal and glycoaldehyde. The concentration of glyoxal and glycoaldehyde was photometrically (Shimadzu UV-VIS spectrophotometer UV-1240) assayed by using the reaction with MBTH as described previously [19].

Determination of 3-deoxyglucosone. The concentration of 3-deoxyglucosone was determined by HPLC (Shimadzu SCL-10AVP fitted with a Shimadzu SPD-10AVP UV-VIS detector) analysis by derivatization using 2,3-diaminonaphthalene [20].

Determination of d-glucose. The commercially available enzyme assay kit, F-kit, was utilized for the determination of d-glucose. The kit determines d-glucose by an enzymatic, spectrophotometric method based on the use of hexokinase, glucose-6-phosphate dehydrogenase, and NADP.

Chemiluminometric detection of O2 generated from the mixture containing carbonyl compounds [21]. MCLA-dependent luminescence was measured with a Luminescence-PSN AB-2200 (Bio-instrument Atto, Japan). The reaction mixture containing Heps-buffered NaCl solution (141mM NaCl, 10mM Heps buffer, pH 7.4), 1mM carbonyl compound, and 20µM MCLA with or without 10mg/ml Hb in a total volume of 200µl was incubated at 37°C for 1h both in the presence and absence of 5mM Tiron. The luminescence was measured for 5min at room temperature. Formed O2 was relatively evaluated as the difference in the luminescence increase between the presence and the absence of 5mM Tiron.

ESR detection of O2 generated from the mixture containing carbonyl compounds. ESR spectra were recorded with a JES-FR30 spectrometer (JEOL, Tokyo). The instrumental conditions were as follows: field set, 335.65mT; scan range, ±5mT; modulation frequency, 100kHz; field modulation width, 0.63 or 100; microwave power, 4mW; microwave frequency, 9.40GHz; and sweep time, 2min. The reaction mixture containing 1mM dl-glyceraldehyde, glyoxal, or d-glucose with or without 10mg/ml Hb in a total volume of 200µl was incubated at 37°C for 1h in the presence of 0.45M DMPO, and ESR spectrum was recorded for each solution.

Determination of O2 generated from carbonyl compounds by WST-1 assay [22]. The reaction mixture (2.0ml) containing 50mM phosphate buffer (pH 7.0), 0.5mM WST-1, and 1 or 5mM carbonyl compounds
was incubated at 37°C in the presence or absence of 5mM Tiron. O₂ was calculated as the difference in the absorbance increase at 438 nm ($\Delta A = 37 \times 10^3$) between the presence and absence of 5mM Tiron.

**Results**

**Effect of addition of carbonyl compound on RBC deformability**

Incubation of RBC with each carbonyl compound lowered the RBC deformability even at low concentration of 1mM (Fig. 1). The decrease rate depended on the kinds of carbonyl compounds, in which both DL-glyceraldehyde and glyoxal were significantly decreased ($p < 0.05$). 3-Deoxyglucosone was not significant, but showed a much more decrease rate than those of glycolaldehyde or D-glucose. In addition, the decrease rate was also different among volunteers, especially glyoxal exhibited a wider range of decrease rate than that of other carbonyl compounds. This result may suggest that the sensitivity for carbonyl compounds differs among volunteers.

**Protective effect of aminoguanidine**

Aminoguanidine inhibited the decrease in deformability by DL-glyceraldehyde or glyoxal (Table 1). Average of the inhibition rate was 44% and 39%, respectively. Using the results of six volunteers, the inhibition effects were not statistically significant. The protective effects were different among volunteers. Especially the result on a volunteer (F) differed from that on the other volunteers (A–E). On its volunteer, no inhibition effect was recognized for the decrease by glyoxal. In addition, on the DL-glyceraldehyde, the incubation with aminoguanidine caused more decrease of RBC deformability than that with DL-glyceraldehyde alone. Using the results of five volunteers except its volunteer, aminoguanidine significantly inhibited the decrease in RBC deformability by DL-glyceraldehyde ($p < 0.05$). On glyoxal, it was not significant, but showed a much more inhibition rate than those of six volunteers (average of inhibition rate; 53%).

**Reactivity of carbonyl compounds with Hb.**

Fig. 2 depicts the remaining amount of carbonyl compounds after 10mg/ml Hb was mixed with 1mM

![Graph of Effect of carbonyl compounds on RBC deformability](image1)

![Graph of Reactivity of carbonyl compounds on Hb](image2)

**Table 1**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volunteers</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-Glyceraldehyde</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>80</td>
<td>75</td>
</tr>
<tr>
<td>Glyceraldehyde alone</td>
<td>75</td>
<td>74</td>
</tr>
<tr>
<td>+Aminoguanidine</td>
<td>80</td>
<td>74</td>
</tr>
<tr>
<td>Glyoxal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>90</td>
<td>82</td>
</tr>
<tr>
<td>Glyoxal alone</td>
<td>86</td>
<td>74</td>
</tr>
<tr>
<td>+Aminoguanidine</td>
<td>91</td>
<td>80</td>
</tr>
</tbody>
</table>

Each value shows the RBC deformability (%).
carbonyl compound for 1 h. Of five kinds of carbonyl compounds tested, the remaining amounts on D,L-glyceraldehyde and glyoxal were significantly decreased ($p < 0.05$). Therefore, it was shown that those carbonyl compounds have higher reactivity with Hb compared with other carbonyl compounds.

Detection of $O_2^\cdot$ generated from the mixture containing carbonyl compounds

We detected $O_2^\cdot$ formed during incubation of reaction mixture by chemiluminescence method using MCLA as chemiluminescent probe. As shown in Table 2, it was impossible to detect the presence of $O_2^\cdot$ in the mixture of Hb and carbonyl compounds. Next, $O_2^\cdot$ was determined during incubation of carbonyl compound alone based on our previous report [22]. As shown in the table, it was possible to detect on four kinds of carbonyl compounds except D-glucose. D,L-Glyceraldehyde exhibited the highest activity for $O_2^\cdot$ generation in the carbonyl compounds tested. Although we tried to detect the presence of $O_2^\cdot$ by the ESR spin trapping method using DMPO as $O_2^\cdot$ detection probe, it was impossible to detect on not only the mixture of Hb and carbonyl compounds but also the carbonyl compound alone. Next, we tried to determine $O_2^\cdot$ formed during incubation of the carbonyl compound alone by WST-1 method. As shown in Table 3, D,L-glyceraldehyde exhibited the highest activity for $O_2^\cdot$ generation and produced 1.5 nmol O$_2^\cdot$/ml/h at 1 mM level.

Effect of $O_2^\cdot$ on RBC deformability

The result shown in Table 3 indicates that RBC was exposed to 1.5 nmol O$_2^\cdot$/ml/h at the maximum when the RBC deformability was measured. Then, we tried to investigate whether $O_2^\cdot$ generated from carbonyl compounds results in the decrease of RBC deformability. When the HPX-XOD system (0.1 mM HPX, 1.25 $\times$ 10$^{-3}$ U/ml XOD; 1.6 nmol/ml O$_2^\cdot$) equivalent to the formation rate by carbonyl compounds was incubated with RBC, the RBC deformability was little changed compared with control (Table 4). Furthermore, we investigated the dose-dependent manner of the XOD on the RBC deformability. Table 5 depicts the dependence of the concentration of XOD on the RBC deformability. As can be seen, no change was observed not only at the concentration of 1.25 $\times$ 10$^{-3}$ U/ml XOD which produced 1.6 nmol O$_2^\cdot$/ml/h, but also at 2.50 $\times$ 10$^{-3}$ U/ml which produced 9.8 nmol O$_2^\cdot$/ml/h. A remarkable decrease (17%) in RBC deformability was observed at the concentration of 4.00 $\times$ 10$^{-2}$ U/ml which produced 196.9 nmol O$_2^\cdot$/ml/h. This result means that the formation rate of 1.5 nmol O$_2^\cdot$/ml generated by carbonyl compounds hardly affected the decrease in RBC deformability caused by addition of carbonyl compounds.

### Table 2

MCLA-derived chemiluminescence (CL) induced by the mixture containing carbonyl compounds

<table>
<thead>
<tr>
<th>Carbonyl compounds (1 mM)</th>
<th>CL for $O_2^\cdot$ (x10$^3$) for 5 min</th>
<th>$+\text{Hb}$</th>
<th>Carbonyl compound alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>D,L-Glyceraldehyde</td>
<td>nd</td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td>Glyoxal</td>
<td>nd</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Glycolaldehyde</td>
<td>nd</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>3-Deoxyglucose</td>
<td>nd</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
</tbody>
</table>

The mixtures of 1 mM carbonyl compound, 20$\mu$M MCLA with or without 10 mg/ml Hb in Hepes-buffered NaCl solution (pH 7.4) were incubated at 37°C for 1 h and measured CL for 5 min. nd means “not detected.”

### Table 3

Estimation of superoxide anion generated by carbonyl compounds based on the WST-1 method

<table>
<thead>
<tr>
<th>Carbonyl compounds (5 mM)</th>
<th>Superoxide anion formation (nmol/ml/h)*1</th>
</tr>
</thead>
<tbody>
<tr>
<td>D,L-Glyceraldehyde</td>
<td>6.4 (1 mM; 1.5)\textsuperscript{2}</td>
</tr>
<tr>
<td>Glyoxal</td>
<td>0.2</td>
</tr>
<tr>
<td>Glycolaldehyde</td>
<td>3.8</td>
</tr>
<tr>
<td>3-Deoxyglucose</td>
<td>0.3</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>nd\textsuperscript{3}</td>
</tr>
</tbody>
</table>

The mixtures of 0.5 mM WST-1, 1 or 5 mM carbonyl compound with or without 5 mM Tiron in phosphate buffer (pH 7.0) were incubated at 37°C for 1 h and the absorbance at 438 nm was measured.

*1 Superoxide anion formation (nmol/ml/h) = (ΔA$_{\text{Tiron}}$ - ΔA$_{\text{Tiron}}$) $\times$ 1/37000 $\times$ 10$^6$ $\times$ 2 $\times$ 1/2.

*2 The value shows superoxide anion formation at 1 mM D,L-glyceraldehyde.

*3 nd means “not detected.”

### Table 4

Effect of superoxide anion on RBC deformability

<table>
<thead>
<tr>
<th>Volunteers</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>83</td>
</tr>
<tr>
<td>O$_2^\cdot$-treated sample</td>
<td>80</td>
</tr>
</tbody>
</table>

Each value shows the RBC deformability (%). After RBC suspension (Hct 3%) containing 0.1 mM HPX and 1.25 $\times$ 10$^{-3}$ U/ml XOD was incubated at 37°C for 1 h, the RBC deformability was measured.

### Table 5

Effects of superoxide anion on RBC deformability

<table>
<thead>
<tr>
<th>Concentration of XOD (U/ml)</th>
<th>Superoxide anion formation (nmol/ml/h)</th>
<th>RBC deformability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>74</td>
</tr>
<tr>
<td>1.25 $\times$ 10$^{-4}$</td>
<td>1.6</td>
<td>71</td>
</tr>
<tr>
<td>2.50 $\times$ 10$^{-3}$</td>
<td>9.8</td>
<td>71</td>
</tr>
<tr>
<td>4.00 $\times$ 10$^{-2}$</td>
<td>196.9</td>
<td>57</td>
</tr>
</tbody>
</table>

RBC suspension (Hct 3%) containing 0.1 mM HPX and a given concentration of XOD was incubated at 37°C for 1 h, the RBC deformability was measured. Superoxide anion formation was determined by WST-1 method.
Discussion

It is generally known that d-glucose is present at 3–6 mM level in blood on the healthy adults. On the other hand, the concentration of glyceraldehyde and glyceraldehyde-3-phosphate in human tissues varies greatly among individuals and investigations and they appear to range in concentration from a few micromolar to less than 100 μM [23,24]. In addition, those carbonyl compounds and 3-deoxyglucosone were so reactive agents as to react with bovine serum albumin much faster in the micromolar range of concentrations than 20 mM glucose [25]. According to some studies [26,27], the level of glycolaldehyde can be considered to be about 2 mM in the physiological conditions [28]. Therefore, the levels of carbonyl compounds (1 mM) used in the present investigation can be considered to be close to the physiological levels.

Several lines of evidence support that dL-glyceraldehyde and glyoxal which significantly reduced the RBC deformability appear to be major precursors of AGEs [29]. Much attention has been paid to glyoxal in order to elucidate the relationship between Maillard reaction and various pathways, because it originates from pathways that have been linked to various pathologies, including DNA oxidation, lipid peroxidation [30], and sugar autoxidation [31]. Recently, it was reported that the level of glyoxal-derived AGEs, a protein modified with glyoxal, in plasma proteins and lenses from diabetic patients was higher than those from normal individuals [29].

dL-Glyceraldehyde is known as the intermediate of glycolysis and the glyceraldehyde-derived AGEs have the salient neurotoxicity for rat primary cultured cortical neurons compared with other carbonyl compounds-AGE, protein modified with glycolaldehyde, glyoxal, and methylglyoxal, which are generated by glycation [32]. Moreover, dL-glyceraldehyde, glyceraldehyde, and glyoxal at 1 mM level inactivated Cu,Zn-superoxide dismutase (SOD) which catalyzes the dismutation of O$_2^-$ into hydrogen peroxide and molecular oxygen [33]. It is clear, therefore, that those carbonyl compounds play an important role in the many pathological processes.

In this investigation, we fixed the incubation time at 1 h because a longer time incubation of RBC suspension caused the decrease in the RBC deformability in spite of no addition of carbonyls. But, the RBC life of normal adults was usually about 120 days. Thus, the 1-h incubation reflects a change at the initial stage of reaction of RBC proteins with carbonyl compounds. As described above, therefore, the finding that the incubation for 1 h caused the decrease in the RBC deformability indicates that carbonyl compounds may have a more remarkable effect on the RBC deformability in physiological condition.

In order to elucidate the mechanism of the decrease in RBC deformability, we investigated whether aminoguanidine which was the inhibitor of AGE formation in Maillard reaction suppresses the decrease in the RBC deformability. Brownlee et al. [34] introduced aminoguanidine, a dicarbonyl trap and inhibitor of AGE formation in Maillard reaction in vitro, as a promising pharmacological tool for inhibiting the Maillard reaction in vivo and thereby delaying or preventing the onset of diabetic complications. Since aminoguanidine itself is an amine compound, it could compete with the target amine of the system used and suppress the generation of reactive intermediate. Because of this reason, aminoguanidine inhibits the protein modification by glyoxal in Maillard reaction [35]. Therefore, the protective effect for the decrease in RBC deformability in the present investigation may have been caused by a competitive inhibition by aminoguanidine on Maillard reaction between RBC proteins and carbonyl compounds.

First stage of Maillard reaction is an electrophilic attack of carbonyl compounds to RBC proteins. Although carbonyl compounds tested have the reactivity with amino groups, the reactivity differs in each kind of carbonyl compound. We selected the Hb as model of RBC proteins attacked with carbonyl compounds and compared its reactivity among five kinds of carbonyl compounds. The concentration of Hb was set to be 10 mg/ml, because the RBC suspension (Hct 3%) can be estimated to have the same concentration of Hb. In order to reproduce the same condition as the determination of the RBC deformability, we incubated the reaction mixture for 1 h.

As already mentioned, dL-glyceraldehyde and glyoxal which lead to decrease the RBC deformability have higher reactivity with Hb compared with other carbonyl compounds. Therefore, it can be considered that the decrease in RBC deformability resulted from the reactivity of carbonyl compounds with Hb.

Although we selected Hb as model of RBC proteins in this investigation, the decrease in RBC deformability may not be considered only by the Maillard reaction between Hb and carbonyl compounds since RBC consists of several kinds of proteins. Especially, RBC membrane proteins play an important role for rheology of RBC because it was reported that RBC deformability was decreased by denaturalization of RBC membrane protein caused by oxidative stress [36]. Therefore, RBC proteins other than Hb should be investigated on the reactivity with these carbonyl compounds hereafter.

The generation of free radicals, such as superoxide anion, by glycated proteins represents one of the major biochemical pathways of oxidative tissue damage and vascular degeneration in diabetes and aging. Uyesaka et al. [36] reported that exposure of RBC to O$_2^-$ caused a marked decrease in RBC deformability with a concomitant increase in cell volume and shape changes. Then, we detected O$_2^-$ formed during incubation of
reaction mixture and investigated the effect of O$_2^-$ on the decrease in RBC deformability. DL-Glyceraldehyde exhibited the highest activity for O$_2^-$ generation of the carbonyl compounds tested and produced 1.5 nmol O$_2^-$/ml/h at 1 mM. It was shown that 1.5 nmol O$_2^-$/ml generated using HPX-XOD system failed to change RBC deformability. These results suggest that O$_2^-$ generated by addition of carbonyl compounds has no involvement in RBC deformability.

In conclusion, carbonyl compounds related to the Maillard reaction decreased the RBC deformability even at 1 mM level of each compound for short reaction time. The decrease rate depended on the kinds of carbonyl compounds, in which both DL-glyceraldehyde and glyoxal significantly reduced ($p < 0.05$) the deformability. The decrease effect with those compounds may be caused by Maillard reaction between several RBC proteins and carbonyl compounds. In addition, O$_2^-$ generated by addition of carbonyl compounds affects the decrease in RBC deformability only to a negligible level.

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References


