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IMPACT OF PROLONGED GLYCATION PRE-TREATMENT ON THE ELECTRON TRANSFER KINETICS OF CYTOCHROME C

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ხანგრძლივი გლიკირების პროცესის გავლენა ციტოქრომ C-ს მუხტის გადატანის კინეტიკაზე

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რეზიუმე

შესწავლილია D-გლუკოზით გამოწვეული ხანგრძლივი (ინკუბაციის დრო: 1-25 დღე) გლიკირების პროცესის გავლენა ციტოქრომ C-ს ჟანგვა-აღდგენით თვისებებზე, 0,1 მ/ლ ფოსფატის ბუფერულ ხსნარებში 200 გ/ლ (1.1მ/ლ) D-გლუკოზას თანაობისას (T=25^{0}C), მოდელირებულ ექსპერიმენტულ პირობებში. ციტოქრომ C-ს და მოდიფიცირებულ ოქროს ელექტროდს შორის ელექტრონული მიმოცვლის კინეტიკა გლიკირების პროცესის სხვადასხვა ეტაპზე გაზომილი იქნა ციკლური ვოლტამპერომეტრიის მეთოდის გამოყენებით. მიღებული ვოლტ-ამპერული მონაცემების ანალიზის საფუძველზე გამოტანილი იქნა დასკვნა, რომ ზემოაღნიშნულ პირობებში გლიკირებული ციტოქრომ C-ს რედოქს აქტივობა შენარჩუნებულია მინიმუმ 25 დღის განმავლობაში.

Introduction. Horse heart cytochrome C (Cyt C), is a small iron-heme protein (12 kDa molecular weight) with a well known molecular structure [1]. As a multi-functional enzyme, it is involved in electron transfer in the mitochondrial respiratory chain, shuttling electrons between the enzymes, Cyt C reductase and Cyt C oxidase, which are both embedded in the mitochondrial membrane [2]. It also plays a major role in the cell apoptosis - the life and death switching of the cell [3]. So, maintaining Cyt C-s native structure and live redox activity is very important for its proper cellular function. Among agents that can affect Cyt C-s native structure the important role plays glycation by the different sugar agents. Later can react with N-terminal and lysyl side chain amino groups of protein with different intensity and speed, hence effect its redox as well as apoptotic activity. Glycation of proteins with glucose, (as well as with glycosal or methilglucosal) in general is a slow process [4,5]. Some authors on the bases of spectral data [5] came to the conclusion that modification of Cyt C with (125 mM) glucose at T=37^0 even after 1 week was relatively insignificant. Prolonged (up to 20 days) glycation of Cyt C with (1.0M) glucose can lead to the change of redox status of Ferricytochrome C to Ferrocytochrome C and finally possibly weaken ability of Cyt C to transport electrons in the respiratory chain [4]. Direct kinetic studies of electron transfer (ET) properties of Cyt C at modified electrodes in buffer solutions containing different concentrations of sugar, revealed that electron transfer rate constant of electron exchange decreases with increasing of glucose concentration in the solution [6]. According to above mentioned effect of sugar content in the solution on Cyt C ET rate was not caused by glycation of protein itself leading to the weakening the ability of Cyt C electron transfer properties, but was explained as the solutions viscosity impact on the direct ET process operated in a friction (viscosity)-controlled regime [6]. Indeed, as it was mentioned above, glycation process of proteins with glucose in general is very slow process [3-5] and during the timetable of experimental kinetic measurements after adding glucose to the solution (several hours) hardly can affect the Cyt C chemical nutshell. However, effect of prolonged glycation of Cyt C on its electron transfer properties have not yet been directly studied. In the present study we investigated effect of glycation process by D-Glucose on the electron transfer properties of Cyt C in more than 3 weeks of incubation period at T=25^{0}C. Assessment of effect of glycation of Cyt C was observed by direct measurement of

kinetics of electron transfer process between freely diffusing Cyt C and the artificial electron transfer partner - gold electrode by different incubational periods. Taking into account that proteins adsorbed onto a bare metal electrode surface, denature due to the strong electrostatic interaction with the surface and losses their activity [6,7], we covered the gold electrode with a modifier 4,4'-bipyridyl, which form an adsorbed layer on the electrode surface and facilitate to the electron-transfer reaction [6]. Earlier a diffusion-controlled quasireversible electron transfer was found between a gold electrode modified with bis(4-pyridyl) disulfide using Cyt-c in aqueous solution [7].

Materials and Methods. Horse heart cytochrome C (Cyt C), 4,4'-bipyridyl, NaH2PO4, Na2HPO4, D-Glucose were obtained from Aldrich and used as received. All solutions were prepared using MilliQ water. Experiments were performed with conventional three-electrode system. 2 mm Ø gold disc sealed in Teflon cylinders (BAS) was used as working electrode, platinum wire and Ag/AgCl/3M NaCl were used as the counter and the reference electrodes, respectively. The working electrode was sequentially polished with Alumina water slurry on a Buehler polishing pad, rinsed with water and modified by immediate dipping the gold electrode in 0,01 M solution of 4,4'-bipyridyl for 30 min. Voltamperometric measurements were carried out with Potentiostat CH Instrument Model 1200B controlled by an external PC under the Windows 98/NT/Me/2000/XP/Vista/7/8. All experiments were performed at T=25^{0}C.

Results and Discussions. Typical cyclic voltametric (CV) curves of reduction and oxidation reaction of freely diffusing Cyt C at BP-modified Au working electrode in phosphate buffer solutions containing 200 G/L (1.1M) glucose at scan rate v=0.1 V/sec are shown in Figure 1.

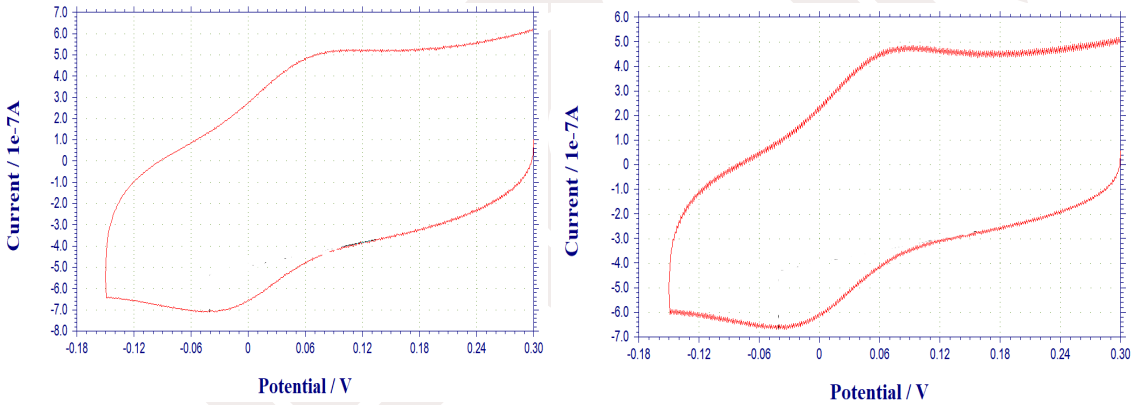

Fig.1 CV curves of Cyt C at Au/BPD modified electrode in 0.1M phosphate buffer (pH 7) containing 4 mg/mL Cyt C in the presence of 1.1M glucose, v=0.1 V/sec, recorded at first day (left) and 25th day (right), T=25^{0}C.

As one can see from Figure1 Cyt C redox response in the solutions containing D-Glucose, recorded at first day and after more than 3 weeks of glycation process, does not differ from each other. Reduction and oxidation peak positions (Ep_{red} and Ep_{oxd}) at the first and last day of experiment are correspondingly (Ep_{red} = -0.042V, Ep_{oxd} = 0.095V) and (Ep_{red} = -0.034V, Ep_{oxd} = 0.083V). Values of the peak to-peak separation- ΔEp ($\Delta Ep = Ep_{red} + Ep_{oxd}$) of electron transfer response of Cyt C in the solution containing 1,1M glucose during 25 days are presented in Figure 1.

Time (days)	1	3	4	7	8	10	11	14	15	16	17	18	21	23	25
ΔEp, mV	136	128	126	130	132	132	117	111	107	107	107	107	114	117	125

Fig.2 shows values of peak to-peak separation, during glycation process observed during 25 days

Note that ΔEp values, evaluated from the experimental cyclic voltammograms, are the measure of the electron transfer activity of redox species and the heterogeneous standard rate constant, k^0, of electron transfer can be determined by the numerically evaluated theoretical relationship between the peak to-peak separation, ΔEp, and the Ψ function using eq 1, according to the method of Nickolson [8].

$$\psi = (RT)^{1/2} k^{o} / (\pi n F D_{o} \upsilon)^{1/2} \quad (1)$$

where n is the number of transferred electrons (here n =1), F is the Faraday constant, Co is the reactant (Cyt C) concentration in the bulk, and D is the diffusion coefficient of the reactant (D =4,91 10-7 cm^2 s^{-1} [6]), T is temperature. Values of rate constant of redox reaction of Cyt C at the different periods of glycation process, calculated from the experimental cyclic voltammograms using equation (1) are presented on figure 3.

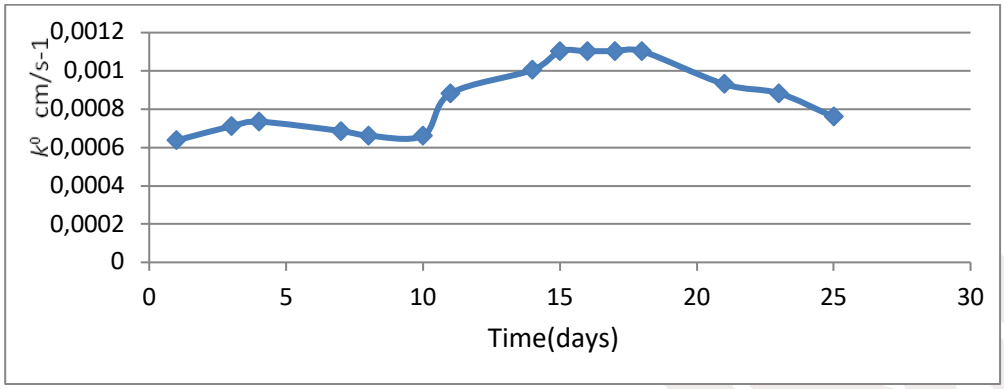

Fig. 3 Values of of rate constant k^0 of Cyt C at different incubation period in 0.1 M phosphate buffer (pH7) containing 1.1M glucose, T= 25 ^{0} C.

As one can see from Fig. 3 electron exchange properties of protein are not damaged by prolonged glycation process (rate constant of electron exchange between glycated protein and electrode even increases during 18 days) and in overall glycated Cytochrome C preserves its redox activity at least during observed period (up to 25 days).

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SUMMARY

The effect of long term (up to 25 days) glycation process by D-glucose on the electron transfer properties of Cyt C under artificial conditions, using modified gold electrode, as a redox partner, has been studied. The redox kinetics of Cyt C, in the 0.1 M buffer solution containing 4 mg/mL Cyt C and 200 G/L D-glucose (T=25 ^{0}C), by the glycation time more than three weeks was directly measured using the method of cyclic voltammetry. Systems voltamperic response has shown, that redox activity of glycated Cyt C is preserved upon the glycation time, at least, during the incubation period up to 25 days.

Keywords: Cytochrome C, Glycation, D-glucose, cyclic voltammetry, electron transfer