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POTASSIUM ASCORBATE AS PROTECTIVE AGENT IN THE OXIDATION OF THE RED BLOOD CELLS

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Free radicals and oxidative substances are involved in many degenerative diseases modifying cell physiological properties. Many mechanisms can be disrupted, including the K-Na channels, with a consequent potassium loss. Free radicals are also able to modify erythrocytes stability leading to peroxidation of the lipid membrane, oxidation of haemoglobin (Hb) and finally to the formation of Heinz Bodies. Such a process can also be induced or enhanced by strong oxidants like acetylphenylhydrazine (APH). In the present work the antioxidant properties of potassium ascorbate acting on the red cells treated with APH are investigated.

1. Introduction

Oxidative processes are involved in many degenerative diseases leading to various cell modifications. Recognised facts that deserve particular attention are the oxidant action exhibited by free radicals (FRs), that is involved in promoting and developing cancer [1-3], and the potassium loss from the cell with the consequent alteration of Na-K pumps. The loss of potassium is strictly connected with apoptosis ([4] and references therein). At normal intracellular K⁺ concentration, about 140-150 mM, the apoptotic mechanisms are dormant due to the inability to activate key protease and nuclease enzymes involved in the apoptotic process. The ubiquitous occurrence of such processes in living cells and tissues makes reasonable to extend the previous considerations to the red blood cells. Due to their systemic character the erythrocytes circulating in the blood stream can reach all the organs and undergo FRs action. The erythrocytes stability can be reduced by peroxidation of the

lipid membrane and oxidation of hemoglobin (Hb). The Hb oxidation process starts with the formation of methemoglobin, continue until endoerythrocytes inclusion called Heinz Body are formed inside the red cell, and ends with the release of hemin [2, 5]. This oxidation pathway can also be induced or enhanced by a strong oxidant like acetylphenylhydrazine (APH).

In the present work we are interested in examining the effects of potassium ascorbate (K-asc) on APH treated erythrocytes collected from healthy volunteers using Mössbauer spectroscopy.

2. Materials and methods

The samples were prepared from venous blood taken from healthy volunteers. Five heparinized tubes (5 ml of blood each one) were collected from each person. One of the five tubes was used as control (C), the other four were incubated in a 30mM APH solution in Sörensen buffer (pH=7.6), with the following procedures : in the first sample (APH50) 5 ml of whole blood were incubated for 50 minutes with APH; in the second (ASC50), third (K50) and fourth sample (KASC50), 5 ml of whole blood were added to APH and mixed with a solution of ascorbic acid, potassium bicarbonate and potassium ascorbate (K-asc), respectively.

K-asc is a salt resulting from the salification of ascorbic acid by potassium bicarbonate, and has antioxidant properties. In this preliminary work the K-asc concentration is based on the potassium concentration present in the cell at physiological condition. Ascorbic acid, potassium bicarbonate and potassium ascorbate were diluted in distilled water to prepare the solutions.

At the end of the incubation time, the samples were centrifuged for 10 minutes at 3000 RPM (~ 1500 g). After removal of the supernatant the cells were resuspended in 0.9% NaCl solution and centrifuged again at 3000 RPM for 10 minutes. The procedure was repeated twice. The packed red cells obtained from the last centrifugation were collected in a Mössbauer sample holder and immediately frozen in liquid nitrogen. The measurements were performed at 77K with a conventional Mössbauer spectrometer (Takes inc.) working at constant acceleration (drive from Wissel Instruments) in transmission mode. The source was $^{57}\text{Co}/\text{Rh}$ with an activity of 3.7 GBq (100 mCi).

3. Results and discussion

The antioxidant action of potassium ascorbate was followed during the incubation with the strong oxidant APH in various environments. Since K-Asc is the reaction product of ascorbic acid (Asc) and potassium bicarbonate (K), the K50 and ASC50 samples were also prepared, as reported in the materials and methods section, in order to observe the action of the two substances separately.

Mössbauer spectra revealed that only oxy-Hb and deoxy-Hb, as expected, are present in the spectrum of the control sample (C - figure 1). The spectra of the treated samples (figure 2) show the disappearance of the Deoxy-Hb subspectra and the formation of a new component characteristic of oxidation products like methaemoglobin and haemichromes.

The increase of this latter oxidation component corresponds to a decrease of the oxy-Hb component (APH50, figure 2A). All the Mössbauer spectra were fitted with two subspectra obtained from the characteristic parameters of oxy-Hb and deoxy-Hb (figure 1) and from oxy-Hb and the envelope of all the oxidation products (figure 2) [5].

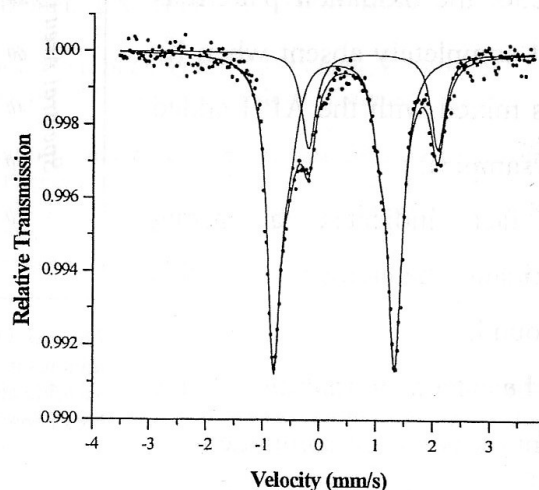


Figure1. Untreated erythrocytes (Control): oxyHb and deoxyHb subspectra are indicated.

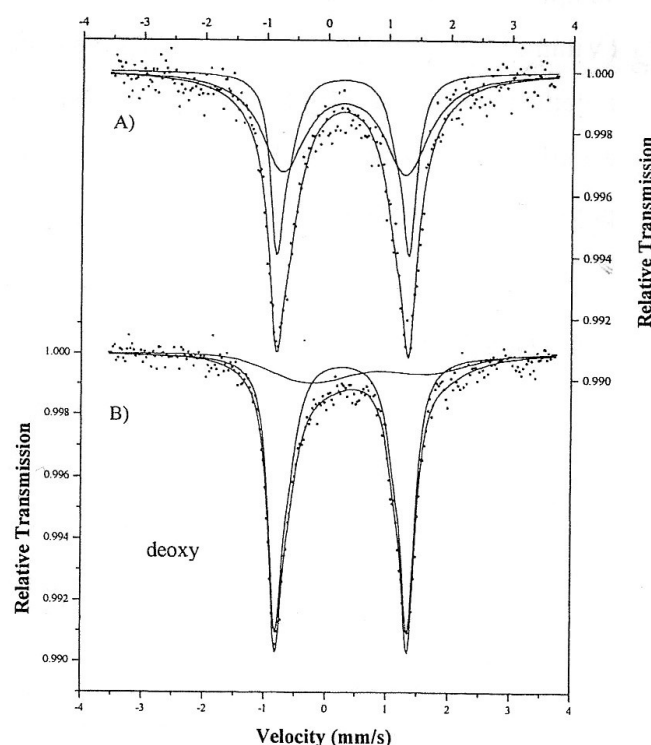


Figure2. Red cells incubated with APH for 50' (A); red cells incubated with APH and potassium ascorbate for 50' (B).

As it can be noted from figure 2B, that represent the spectrum of KASC50, the oxidation pattern is almost completely absent when K-Asc is mixed with the APH added blood samples.

This fact indicates a strong antioxidant behaviours of this compound.

it can be calculated that the relative amount of oxidation components is about 60% after 50 minutes of

incubation with APH, while in the KASC50 sample the action of potassium ascorbate reduces the oxidation to about 20%. The percent area of the oxidation product of all the samples (figure 3) confirms once more the antioxidant behaviour of ascorbic acid (Vitamin C). Our results show that potassium ascorbate too has antioxidant behaviour, together with the ability to carry potassium inside the red cells. As reported in [6], in fact, ascorbic acid bound to K^+ can be transported across the membrane maintaining the physiological potassium concentration.

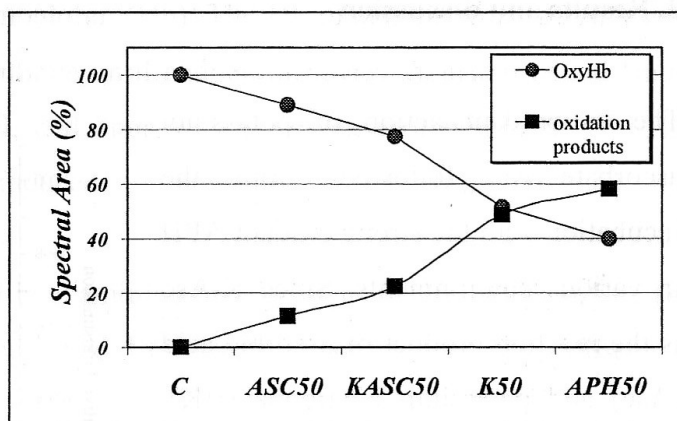


Figure3. Oxidation products in the erythrocyte samples after 50 minutes incubation with APH alone (APH50) and together with ascorbic acid (ASC50), potassium ascorbate (KASC50), potassium bicarbonate (K50).

References

- [1] M.R.Clemens, Klin. Wochenschr 69 (1991): 1123-1134;
- [2] P. Jarolim, M.Lahav, S.C. Liu, J. Palek, Blood 76 (1990) : 2125-2131
- [3] B. Halliwell, Nutr. Rev. 52 (1994) 253-265;
- [4] F.M. Hughes Jr, C.D. Bortner, G.D. Purdy, J.A. Cidlowski, J. Biol. Chem. 272 (1997) 30567-30576
- [5] S.Croci, I.Ortalli, G.Pedrazzi, G.Passeri and P.Piccolo, Hyperfine Interaction 126 (2000): 47-52
- [6] W. Lohman, Biophys-Struct-Mech. 184; 10(4):205-10