PRODUCTION OF GAS PHASE SUPEROXIDE RADICALS BY THERMOSTABLE COMPLEXES FROM RAISIN AND GRAPE SEEDS

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Superoxide (O_2^-)-producing thermostable complexes of NADPH-containing protein component (NPC) with Fe(III) – NPC-Fe(III) was isolated and purified from pink raisin (without seeds) and grape seeds for the first time. In particularly, during isolation and purification of these complexes its fractionation at pH 9,5, precipitation at pH 4,8, ion exchanging chromatography on cellulose DE-52 and incubation in boiling water were carried out. The forms and maximums of optical absorption specta of these complexes from grape seeds significantly differs from that of the complex from raisin. But in both cases in UV region the characteristic for the proteins absorptions at 280 nm were observed. The arithmetic averages of produced O_2^- in solution and gase phase were determined. In a lyophilized state these complexes provide high stability, when losing O_2^--producing activity only to 11-12% during storage a year. After blowing of the aqueous solution of these complexes by oxygen, the monocomponent and regulating stationary concentration of the gas phase superoxide radicals were produced and transferred with oxygen into glass or silicone tubes. In the perspective the produced gas phase O_2^- can be used at lung infection diseases with the oxygen mask.

Keywords: *gas phase superoxide radical, complex, raisin, grape, seed.*

There is a certain antioxidant activity in the tissues of raisins and grape seeds, they exhibit protective effect at different types of pathological states and diseases [1-4]. As active intermediates, raisins and grape seeds contain ions of various metals, in particular, iron and copper [5,6]. On the other hand, the antioxidant status of raisin and grape seed should be in physiological balance with the prooxidant status, which should be connected with Fe(III) and Cu(II) ions.

The aim of this work is to isolate and purify NPC -Fe(III) thermostable complex from pink raisin and white grape seeds and use these biosystems as sources of gas phase O_2^-.

Material and methods.

Isolation and purification of isoforms of the NPC-Fe (III) complexes from grape raisin and grape seeds.

The isoforms of O_2^- - producing NPC-Fe (III) complexes from grape raisin and grape seeds isolated and purified by licensed methods [7], using process of the releasing from indicated sources of the isoforms of NPC-Fe(III) complexes at pH 9,5 by ferriHb (50 mkM), its precipitation at pH 4,8 and solubilization in the water at pH 9,5. The further process of purification of the fractions of these complexes includes ion-exchanging chromatography on cellulose DE-52 and gel filtration on Sephadex G-100 at pH 9,5. Then, for the removing of the possible traces of other proteins, the thermal treatment of the aqueous solutions of indicated above complexes by its heating in boiling water, during 10-12 min and centrifugation were carried out.

Determination of NADPH in NPC-Fe (III) complex. NADPH group in the NPC-Fe (III) complex was determined by spectrofluorimetric method, using Perkin Elmer spectrofluorimeter (USA). The emission peak of NADPH group as a part of NPC was registered at 430 nm with the 370 nm excitation length [8].

Determination of the Fe(III) in the composition of isoforms of O_2^--producing complexes.

Using the ortophenantroline optical spectral method [9] the content of Fe(III) in these complexes was determined only after separation of Fe(III) by EDTA and reduction of Fe(III) by sodium dithionite.

Electrophoresis of the obtained complexes and hybrid associates was performed in 10% polyacrylamide gel (PAAG) for the proteins of acidic and basic

character. The specific content (mg/g) of NPC-Fe(III) was determined by weighing after its desalting and vacuum lyophilization.

Determination of the stationary concentration of produced O_2^- in gas phase.

The gas phase O_2^- was generated during blowing of the aqueous solutions of these complexes by 0,1 atm oxygen at 10 min, in room temperature. As a control, the optical absorbance of adrenochrome, forming during oxidation of the solution of adrenaline only by oxygen in similar conditions was used.

The stationary concentration of the produced O_2^- in moles (M) was determined by the adrenaline method, calculating the ratio of the density of the maximum optical absorption (A) of the formed adrenochrome (at 500 nm) to the molar absorption of the adrenochrome (E = 750 M^{-1} cm^{-1}): A/E, taking into account that 1M adrenaline is oxidized by 1M of O_2^- [10].

Results and discussion. During ion exchange chromatography of NPC-Fe(III) complex from raisin and grape seeds in the column of cellulose DE-52 at pH 9,5 these biosystems don't linger in the cellulose. After concentration and gel-filtration of the complexes NPC-Fe(III) in the column of Sephadex G-100, the primary fractions of NPC-Fe(III) was collected with symmetrical elution chart. After joined of these fractions together, its incubation in boiling water to 10-12 min were carried out for the removing of the possible traces of other proteins.

During electrophoresis in 10% PAAG the above mentioned complexes NPC-Fe(III) don't pass through the tube with PAAG, they are aggregated at the entrance of this gel. However, after staining PAAG for water soluble acidic and basic proteins, accompanying of NPC-Fe(III), were not detected.

Thus, on the base of symmetrical elution chart of NPC-Fe(III) through G-100, absence of coloring strip for acidic and basic water-soluble proteins on PAAG, not changed value of optical-spectral index (A_{280}/A_{430}) for NPC-Fe(III) complex from raisin and seeds, and removing the possible traces of the proteins (including antioxidant ferments) by thermal treatment, we can indirectly speak about the purify of these complexes. The optical absorption spectra of NPC-Fe(III) complexes from raisin and grape seeds were presented in Fig. 1.

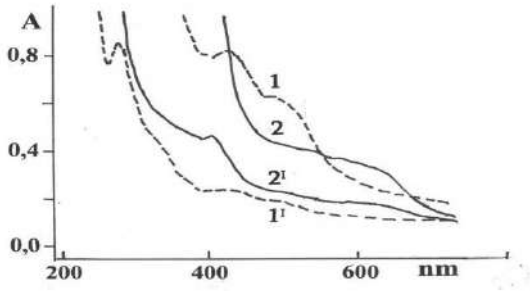

Fig.1. Optical absorption spectra of slightly opalescence aqueous solution of NPC-Fe(III) complex from raisin (1, 1^{I}) and grape seeds (2, 2^{I}) at pH 9,5.

As indicated in Fig.1, the forms of the optical absorption spectra of the isoforms of NPC-Fe(III) from raisin and grape seeds were significantly differed in visible region. However, in UV region the characteristic for the protein absorption at 280 nm were observed. The obtained NPC-Fe(III) complexes produce O_2^- using Fe(III) as electron bridge from NADPH in its composition to molecular oxygen, reducing it up to O_2^-. The latters oxidize adrenaline up to adrenochrome, and these processes are suppressed under the influence of $2 \cdot 10^{-8}$M Cu,Zn-СОД .

The determined quantitative indices of the NPC-Fe(III) complexes from raisin and grape seeds is presented in Table.

Table

The arithmetic averages of specific amounts of Fe(III), fluorescence intensity of the NPC-Fe(III) – «F» in relative units, the specific amount of the NPC-Fe(III) and stationary concentration of produced O_2^- in solution and gas phase (n=6).

Sources of NPC-Fe(III)	Fe(III) mkg/mg	«F» (fluorescence intensity)	Specific amount of the NPC-Fe(III) (mg/g)	O_2^- (mkM) in solution	Gas phase O_2^- (mkM)
Grape raisin	1,8	110,3	52,3	4,3	3,8
Grape seeds	2,3	121,1	58,8	5,4	4,9

As indicated in Table the quantitative indices of NPC-Fe(III) from grape seeds were higher in comparison with indices of raisin.

During heating of these NPC-Fe(III) complexes in boiling water during 10-12 min the any denaturing effect of these complexes doesn't observed. Thus the isoforms O_2^--producing complexes from grape seeds and raisin are high thermostable biosystems. The higher termostability of these O_2^--producing complexes can be connected with the pulsating increase of the temperatures to 280-300°C, during nanoseconds, for transmission of the redox metabolic processes [11].

Thus, there are some perspectives for using NPC-Fe(III) complex from raisin and, especially, from grape seeds, as energetic, natural and relatively stable (thermostable) system for continuously production of O_2^-, which are known as a bactericidal and antiviral agents [12].

As we have already mentioned above, these NPC-Fe(III) complexes in lyophilized state, especially in the nitrogen atmosphere, practically don't lose their O_2^--producing activity when kept at -10-15° throughout the year.

In fact, the oxygen isstabilized the gas phase O_2^- and it is possible by forming of coordination band between O_2 and O_2^-. On the other hand, it is known, that the gas phase O_2^- is formed 1.in air by reducing the molecular oxygen with negative metal ions traces, 2. by electrochemical way, 3.during the influence of the earth crust radioactivity and during photosynthesis within plant [13-15].

It is concluded, that O_2^- producing complexes NPC-Fe(III), isolated from pink raisin and white grape seeds, are new vigorous, natural and continuously superoxide producing (prooxidant) agents. The produced gas phase O_2^- from raisin and grape seeds NPC-Fe(III), in perspective, can by used with the oxygen mask at lung infection diseases.

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ՔԻՇՄԻՇԻՑ ՈՒ ԽԱՂՈՂԻ ԿՈՐԻԶՆԵՐԻՑ ԱՆՋԱՏՎԱԾ ՋԵՐՄԱԿԱՅՈՒՆ ԿՈՄՊԼԵՔՍՆԵՐՈՎ ԳԱԶ ՖԱԶԱՅԻՆ ՍՈՒՊԵՐՕՔՍԻԴ ՌԱԴԻԿԱԼՆԵՐԻ ԳՈՅԱՑՈՒՄԸ

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Առաջին անգամ խաղողի քիշմիշից (առանց կորիզների) և խաղողի կորիզներից անջատվել և մաքրվել են սուպերօքսիդ ռադիկալներ (O_2^-)-գոյացնող ջերմակայուն կոմպլեքսներ՝ կազմված ՆԱԴՐԻ-պարունակող սպիտակուցային բաղադրամասից (ՆՍԲ) և Fe(III)-ից՝ ՆՍԲ–Fe(III): Այս կոմպլեքսների անջատման ու մաքրման ընթացքում մասնավորապես իրագործվել են դրանց ֆրակցիոնացումը pH 9,5-ում և նստեցումը pH 4,8 –ում, իոնափոխանակային աբսորբումը DE-52 ցելյուլոզի վրա և ինկուբացումը եռացող ջրում:

Խաղողի կորիզներից ստացված կոմպլեքսների օպտիկական սպեկտրների ձևը և մաքսիմալ կլանումները էապես տարբերվում են քիշմիշից ստացված կոմպլեքսների համանման չափանիշներից: Սակայն երկու դեպքում էլ ՈւՄ մարզում նկատվում են սպիտակուցներին բնորոշ կլանումներ 280 նմ-ում: Որոշված են արտադրված O_2^- միջին թվաբանական արժեքները լուծույթում և գազ ֆազում: Այս կոմպլեքսները լիոֆիլիզացված վիճակում ցուցաբերում են բարձր կայունություն՝ կորցնելով O_2^- -գոյացնելու ակտիվությունը ընդամենը 11-12% մեկ տարվա ընթացքում: Այս կոմպլեքսների ջրային լուծույթների մեջ թթվածին փչելով արտազատվում են մոնոկոմպոնենտ, կարգավորվող ստացիոնար քանակ-

ներով գազ ֆազային սուպերօքսիդներ՝ թթվածնի հետ միասին, և տեղափոխվում են սիլիկոնային կամ ապակյա խողովակներով: Հեռանկարում այս կերպ բնական աղբյուրից գեներացված գազ ֆազային սուպերօքսիդները կարելի է օգտագործել թոքային ինֆեկցիոն հիվանդությունների ժամանակ՝ թթվածնային դիմակի հետ համակցված:

Բանալի բառեր՝ *գազ ֆազային սուպերօքսիդ ռադիկալ, կոմպլեքս, քիշմիշ, խաղող, կորիզ:*

ПРОДУЦИРОВАНИЕ ГАЗОФАЗНЫХ СУПЕРОКСИДНЫХ РАДИКАЛОВ ТЕРМОСТАБИЛЬНЫМИ КОМПЛЕКСАМИ ИЗ КИШМИША И КОСТОЧЕК ВИНОГРАДА

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Впервые выделены и очищены супероксид (O_2^-) - продуцирующие, термостабильные комплексы между НАДРН содержащим белковым компонентом (НБК) и Fe(III) – НБК-Fe(III) из кишмиша (без косточек) и косточек винограда. В частности, во время выделения и очистки этих комплексов были осуществлены фракционация в pH 9,5, осаждение в pH 4,8, ионообменная хроматография на целлюлозе DE-52 и инкубация в кипящей воде. Формы и максимумы спектров оптических поглощений этих комплексов из виноградных косточек существенно отличаются от комплексов из изюма. Но в обоих случаях в УФ регионе наблюдались характерные для белков поглощения при 280 нм. Определены средние арифметические величины продуцируемых O_2^- в растворе и в газовом фазе. В лиофилизованном состоянии эти комплексы оказывают высокую стабильность, в то время как теряют O_2^-продуцирующую активность только на 11-12% при хранении год. После продувания кислородом водных растворов этих комплексов, продуцируются монокомпонентные газофазные супероксидные

радикалы с регулируемой стационарной концентрацией и передаются с кислородом через стеклянную или силиконовую трубки. В перспективе продуцированные газофазные O_2^-могут использоваться с кислородными масками при легочной инфекции.

Ключевые слова: *газофазный супероксидный радикал, комплекс, изюм, виноград, косточка.*

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