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ISOLATION AND PURIFICATION OF SUPEROXIDE-PRODUCING PROTEIN COMPLEX FROM HELIANTHUS TUBEROSUS, DAUCUS SATIVUS, AND SOLANUM TUBEROSUM

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ABSTRACT

In this study, isoforms of NPC-Fe(III) complexes were isolated and purified from Armenian Helianthus tuberosus, Solanum tuberosum, and Daucus sativus. The physicochemical properties of these complexes were determined for the first time, including specific amounts, O_2^--producing activity, stationary concentration of O_2^-, and fluorescence intensity. The complexes exhibit maximal optical absorptions in the visible and UV regions. Hybrid associates between the isolated NPC-Fe(III) complexes and NADPH oxidase (Nox) were formed, and the production of O_2^- by these hybrid associates was observed. The complexes continuously produce O_2^- in aerobic conditions by utilizing electrons from NPC, transferring them to Fe(III), and subsequently reducing O_2 to O_2^-. Finally, O_2 stabilizes O_2^-.
Overall, these results suggest that the isoforms of NPC-Fe(III) complexes represent new prooxidant components in the aforementioned sources and that NPC serves as a bioelectric source for the production of O_2^-.

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Introduction

Daucus sativus has been shown to exhibit antioxidant activity and, during seasons of lower rainfall, it accumulates phenolic compounds and vitamins [1, 2]. The antioxidant capacity of Solanum tuberosum and Helianthus tuberosus is also known [3, 4]. However, no information was available regarding the prooxidant capacity of the aforementioned root crop foods [5]. On the other hand, the physiological balance between anti-oxidant and prooxidant systems can be observed in aerobic organisms, including the mentioned root crop foods [6, 7]. Additionally, the prooxidant systems, specifically the isoforms of O_2^--producing complexes between NADPH-containing protein component (NPC) and Fe(III) – NPC-Fe(III), have been isolated and purified from various plant systems, also for the first time [8-10].
The objective of this investigation is to isolate, purify, and determine the properties of the O_2^--producing complexes NPC-Fe(III) from Armenian Helianthus tuberosus, Daucus sativus, and Solanum tuberosum [11, 12].

Materials and Methods

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Isolation and Purification of the Isoforms of O_2^--Producing NPC- Fe (III) Complex from Helianthus tuberosus, Daucus sativus, and Solanum tuberosum

The isoforms of O_2^-- producing NPC-Fe(III) complexes from Helianthus tuberosus, Daucus sativus, and Solanum tuberosum (50-100 g) were isolated and purified by licensed method [8]. In particular, the study involved the release of the total fraction of NPC-Fe(III) complexes at pH 9.5 in the presence of ferriHb (50 μM), the precipitation of these complexes at pH 4.8, and their solubilization in water at pH 9.5. The further process of purification of these complexes included ion-exchanging chromatography on cellulose DE-52 and gel filtration on Sephadex G-100 or G-200 at pH 9,5. Then, for the removal of other protein traces, the thermal treatment of water solutions of the aforementioned complexes was performed by heating them in boiling water for 10–12 minutes. The removal of possible residues was performed by centrifugation. The NPC-Fe(III) fractions were eluted with a symmetrical elution diagram and after deionization, the isoforms of the NPC-Fe complex were subjected to vacuum lyophilization. After weighing, the isoforms of the prepared NPC-Fe(III) complexes were stored under anaerobic conditions at -10ºC.

Electrophoresis of the isoforms of NPC-Fe(III) complex was carried out on 7% or 10% polyacrylamide gel (PAAG) for proteins of acidic or basic characteristics.

Determination of NPC in the Composition of Isoforms of NPC-Fe(III) Complexes from Helianthus tuberosus, Daucus sativus, and Solanum tuberosum

NADPH components (NPC), connected with protein, in the composition of isoforms of NPC-Fe(III) complexes Helianthus tuberosus, Daucus sativus, and Solanum tuberosum were determined by measuring fluorescence intensity (F) of the NPC-Fe(III) or NPC in relative units at 450-460 nm with excitation, at 370 nm.

Isolation of NPC From isoforms of Aqueous Solutions of NPC-Fe(III) Complexes from Helianthus tuberosus, Daucus sativus, and Solanum tuberosum

NPC from aqueous solutions of indicated above complexes isolated after its incubation with 10^{-4}M EDTA, for10 min at room temperature, and then ion-exchanging chromatography on the column of DE-52 cellulose at pH9,5. In these conditions,NPC easily eluted from this cellulose. Fe(III) is adsorbed on the cellulose by connection with EDTA. Fe(III) was determined by the known orthophenanthrolein method. Isolated NPC only has reductive properties.

Determination of the Units of O_2^--Producing Activities of These Complexes

O_2^--producing activity units of these complexes from Helianthus tuberosus, Daucus sativus, and Solanum tuberosum were determined by measuring the increased absorption of adrenochrome (at 500 nm) to 50%. The units of specific activities of these complexes are U/mg.

Determination of Stationary Concentrations of O_2^-, Produced by the Isoforms of NPC-Fe(III) Complexes from Helianthus tuberous, Daucus sativus, and Solanum tuberosum in Aqueous Solutions

Adrenaline technique was used to evaluate the maximal optical absorbance of adrenochrome (at 500 nm), which is created during the oxidation of adrenaline by produced O_2^-. Stationary concentrations of O_2^-, produced by isoforms of NPC-Fe(III), were also determined [13]. The molar extinction (E) of the produced O_2^- is up to 750 M-1см-1. The stationary concentrations (M) of O_2^-, produced by these NPC-Fe(III) associates, were determined in homogeneous phase (in solution) by determining the value of A500/E [14, 15]. The optical absorbance of adrenochrome, formed during the oxidation of adrenaline only by the oxygen, was used as a control [16].

Generation of Gas Phase O_2^- by Isoforms of NPC-Complex from Helianthus tuberosus, Daucus sativus, and Solanum tuberosum

After blowing off the aqueous solutions of these complexes at pH 9,5 by oxygen (0,1 atmosphere) at various times the produced O_2^- was transferred with oxygen through glass or silicone tubes (1m or more) [10]. The stationary concentration of O_2^- was determined using the above-mentioned adrenaline method.

During the investigation, the cellulose DE-52 («Whatman», England), Sephadex G-100 or G-200 («Pharmacia», Sweden), adrenaline («Sigma», USA), the spectrophotometer «Cary 60» and spectrofluorimeter «Cary Eclipse» (USA), centrifuge K-70D and K-24 «Janetzki» (Germany) were used.

The isolation of the isoforms of NPC-Fe(III) complexes was carried out six times to check the reproducibility of experiments, as well as to determine the arithmetic mean values.

Results and Discussion

During electrophoresis of the isoforms of O_2^--producing NPC-Fe(III) complexes on PAAG its aggregation on the exit of the PAAG-containing tubules was observed.This appearance was conditioned by aggregation of the isoforms of NPC-Fe(III) on a heterogeneous phase (on PAAG) under the influence of electricity. However, the presence of water-soluble proteins exhibiting acidic and basic characteristics, which were stained with amidoblack, was not detected on the polyacrylamide agarose gel

(PAAG) tubes. This is the first indirect factor that shows how pure the NPC-Fe(III) complexes' isoforms are. The second factor of purity is a symmetrical eluting diagram from G-100 or G-200 Sephadex.The third factor is the invariance of the relation A_{280}/A_{420} during further purification of NPC-Fe(III).

The high thermostability of the isoforms of these NPC-Fe(III) complexes from Helianthus tuberosus, Solanum tuberosum, and Daucus sativus can be connected with pulsate rise in temperature up to 280-300ºC, during nanoseconds, for transmission of redox metabolic processes [15].

The optical absorption spectra of the isoforms of O_2^--producing NPC-Fe(III) complexes from Helianthus tuberosus, Solanum tuberosum, and Daucus sativus with opalescence aqueous solution at pH 9,5 were presented in **Figure 1**.

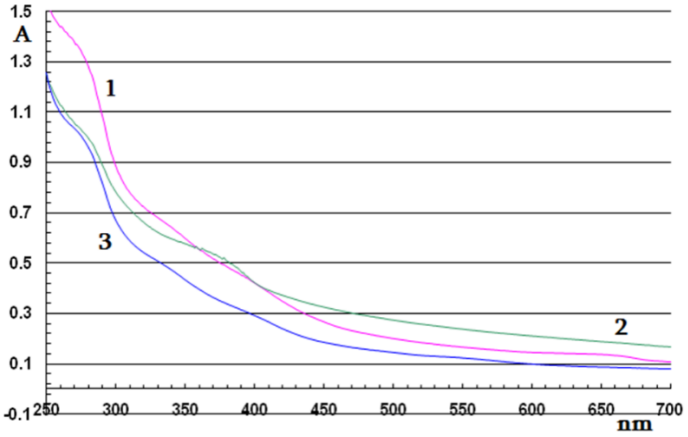

Figure 1. The optical absorption spectra of the aqueous solution of the isoforms of O_2^--producing NPC-Fe(III) complexes from Helianthus tuberosus (1), Solanum tuberosum (2) and Daucus sativus (3) at pH 9,5.

As shown in **Figure 1**, the characteristic maximal absorbance for the proteins in 280 nm is observed. In the visible region, the weekly absorbance at 420 nm, 480 nm, and 520 nm were observed.

The spectrofluorimetric indices presented above O_2^--producing complexes from Helianthus tuberosus (1) Solanum tuberosum and Daucus sativus (3) at pH 9,5 are presented in **Figure 2**.

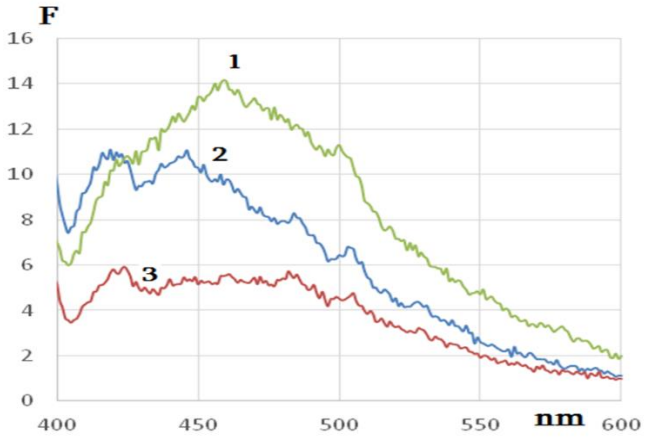

Figure 2. Spectrofluorimetric indices of the isoforms of O_2^-- producing NPC-Fe(III) complex (mg/ml) from Helianthus tuberosus (1), Solanum tuberosum (2) and Daucus sativus (3) at 450-460 nm by excitation at 370 nm, pH 9,5. The «F» is opalescence intensivity in relative units.

The similar spectrofluorimetric indices of NPC isoforms, isolated from root crops of Helianthus tuberosus, Solanum tuberosum, and Daucus sativus were observed. On the other hand, the NPC, as a source of electrons, reduced the potassium permanganate and inhibited the oxidation of adrenaline to adrenochrome.

Some physicochemical indices of the isoforms of NPC-Fe(III) from Helianthus tuberosus, Solanum tuberosum, and Daucus sativus are presented in **Figures 3a-3d**.

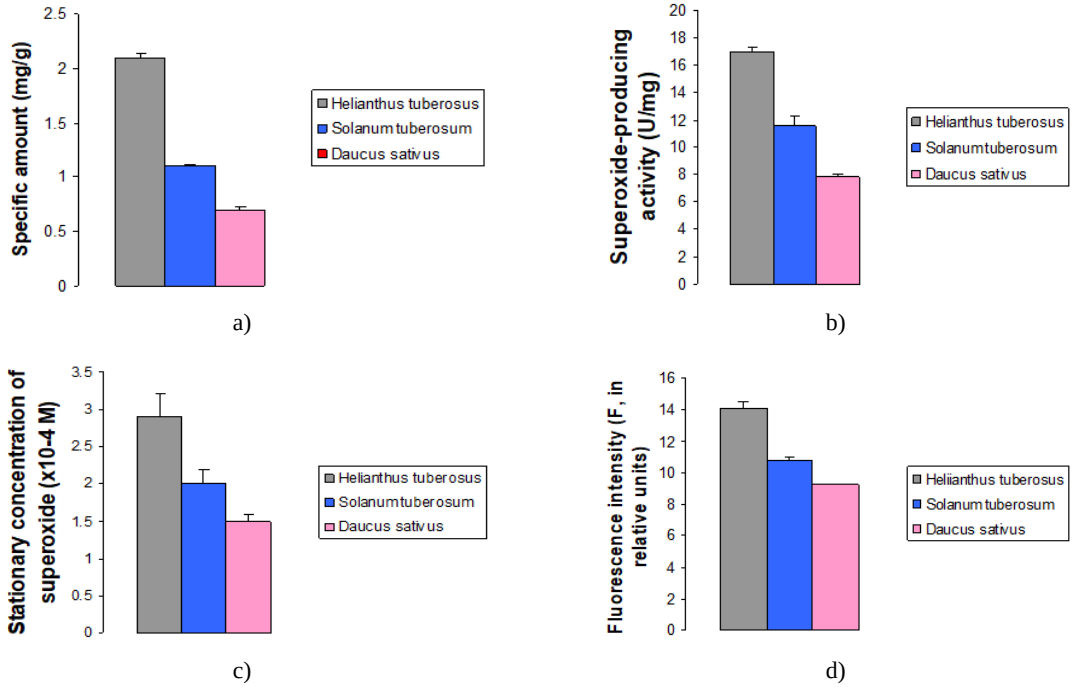

Figure 3. a) The specific contents of isoforms of NPC-Fe(III) from Helianthus tuberosus, Solanum tuberosum, and Daucus sativus. b) The superoxide-producing activities of the isoforms of NPC- Fe(III) from Helianthus tuberosus, Solanum tuberosum, and Daucus sativus. c) The stationary concentration of produced O_2^- by isoforms of NPC-Fe(III) from Helianthus tuberosus, Solanum tuberosum, and Daucus sativus. d) The fluorescence intensity of the aqueous solutions of the isoforms of NPC-Fe(III) from Helianthus tuberosus, Solanum tuberosum, and Daucus sativus.

The NPC, isolated from these complexes (Helianthus tuberosus, Solanum tuberosum, and Daucus sativus), can act as a substrate for the Nox from erythrocyte and leukocyte membranes. Thus, Nox, as a substrate, can be not only free NADPH but NADPH connected with a protein component (NPC), also. Isolated NPC indicates only reductive properties (reduces $KMnO_4$).

The «hybrid associates» – hNPC-Nox produce O_2^- continuously (during 48-72 hours or more at room temperature, only in aerobic conditions), as O_2^-- producing associates – NLP-Nox (NLP is NADPH containing lipoprotein, localized in the external layer of biomembranes) [8]. Nox from biomembranes is localized on the surface of the biomembrane [16].

The O_2^-- producing NPC-Fe(III) complexes, isolated from Solanum tuberosum, Daucus sativus, and Helianthus tuberosus, as well as «hybrid associates» (hNPC-Nox) and separated from these complexes. NPC are not denatured after vacuum lyophilization and during storage under anaerobic conditions (-10ºC) for two years and practically do not lose activity.

Thus, the fundamental significances of obtained results are: 1) in Helianthus tuberosus, Solanum tuberosum, and Daucus sativus the physiological balance between antioxidant system and corresponding prooxidant system (the isoforms of O_2^--producing NPC-Fe(III) complexes) are present, 2) the produced gas phase O_2^- by Helianthus tuberosus, Solanum tuberosum Daucus sativus complexes were stabilized by O_2 and transferred into chemically neutral (glass and silicone) tubes.

The practical significance of obtained results are:1) the use of the liquid phase O_2^-, produced by these complexes in biochemistry for determination of the influence of these O_2^-, as a biological, advantage, comparatively purity, thermostable, continuously acting and easily regulating agent on various biosystems (enzymes, proteins, lipoproteins, biomembranes, DNA etc), 2) in food chemistry, the quantitative and qualitative changes of these complexes, as new estimation of foods quality, can be used , 3) the gas phase O_2^-, produced by these complexes can be used with oxygen mask for treatment of lung infection diseases, 4) liquid and gas phase O_2^-in effective concentrations, as a factor of the proliferation and apoptosis of the cells, microorganisms can be used, 5) by using of the presented above universal method, the preparation of the isoforms of O_2^--producing NPC-Fe(III) complexes from Armenian Helianthus tuberosus, Solanum tuberosus and Daucus sativus, NPC from these complexes, as well, O_2^--producing hybrid associates between NPC-Nox in lyophilized state for commercial aims can be presented, 6) it is possible the use of these NPC in the treatment of immunodeficiency, as a stimulator of the O_2^-- producing activity of immune cells membranes (leukocyte, erythrocyte membranes), 5) it is possible to produce these NPC-Fe(III) isoforms commercially.

Conclusion

Thus, isoforms of a new prooxidant component (the O_2^- -producing NPC-Fe(III) complexes), NPC, and hybrid associates between NPC and Nox (hNPC-Nox) were isolated and purified from Armenian Helianthus tuberosus, Solanum tuberosum, and

Daucus sativus. Their physicochemical properties and mechanisms of influence were determined, and the fundamental and practical significances of the presented data were observed for the first time.

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Conflict of interest: None

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Ethics statement: The experimental procedures followed the guidelines outlined in the European Communities Council Directive (2010/63/UE) and were approved by the Ethics Committee of the Yerevan State Medical University after Mkhitar Heratsi (N10-2/22-IRB APPROVAL, May 19, 2022).

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