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Isolation and purification of superoxide dismutase from cultivated plant cells

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Abstract

Superoxide dismutase (SOD) is among the key enzymes that compose antioxidant enzymatic systems of living organisms. Together with other antioxidant enzymes (catalase, myeloperoxidase, paraoxonase, glutathione peroxidase, etc.), this compound protects aerobic organisms from constantly generated highly reactive toxic oxygen radicals, and thus plays the key role in combating oxidative stress. To date, the role of toxic active oxygen forms in development of more than 100 pathologies has been confirmed. The studies of molecular mechanisms of the reactions involving active forms of oxygen have indicated the need for development of novel antioxidant preparations intended for use in medicine, pharmaceuticals and cosmetics. Since SOD is one of the most powerful natural antioxidants, this enzyme is of great interest to researchers. It can be used in practical medicine for purposes of reducing adverse effect of free radicals and decreasing oxidative stress in cells. Thus, the search for available sources of raw materials for isolating antioxidant substances still remains an actual problem as well as the subsequent design of new medicinal preparations on the basis of these substances for use in combination with basic therapy. The aim of the present work was development and optimization of isolation and purification techniques for superoxide dismutase from medicinal plants (serpentlike rauwolfia, Rauwolfia serpentine (L.) Benth. from the family Apocynaceae, and ginseng Panax Ginseng C.A. Mey), which are constantly stored in the collection of plant tissue cultures of Saint Petersburg State Chemical Pharmaceutical University. For comparison, we have used both the traditional methods and methods that are employed in preparation of high-purity proteins and enzymes that contain metal ions as prosthetic groups. Metal affinity chromatography (IDA-sepharose 6B sorbent) was used to prepare plant SOD with degree of purification 128-131; the yield reached 81%.

References

- [1] I. Fridovich. Biological effects of the superoxide radical. Arch. Biochem. Biophys. 1986. Vol.247. P.1-11.
- [2] I. Fridovich. Superoxide radical and Superoxide dismutase. Annu. Rev. Biochem. 1995. Vol. 64. P.97-112.
- [3] I. Fridovich. Superoxide dismutase anion radical, Superoxide dismutases, and Related Matters. J. Biol. Chem. 1997. Vol.272. P.18515-18517.
- [4] B. Halliwell. Free radicals, antioxidants and human disease: curiosity, cause, or consequence. *Lancet*. 1994. Vol.344. P.721-724.
- [5] B.P. Sharonov, I.V. Churilova. Oxidation of superoxide dismutase with hypochloride. The appearance of isomers with catalytic activity. Reports of the Academy of Sciences USSR. 1990. Vol.314. No.6. P.1500-1502. (russian)
- [6] V.E. Volykhina, E.V. Shafranovskaya. Superoxide dismutase: structure and properties. Bulletin of *VSMU(RU)*. **2009**. Vol.8. No.4. P.6-12. (russian)
- [7] A.V. Maksimenko. Modified preparations of superoxide dismutase and catalase for the protection of the cardiovascular system and lungs. Successes sovr. Biol. (RU). 1993. Vol.113. No.3. P.351-365. (russian)
- [8] A.V. Maksimenko Covalent modification of superoxide dismutase subunits chondriotine sulfate. Biochemistry (RU). 1997. Vol.62. Iss.10. P.1359-1363. (russian)

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- [9] Yu.I. Drozdova. Isolation and study of the properties of human superoxide dismutase recombinant yeast strain Saccharamyces cerevisiae. Author's abstract of dis. ... cand. biol. sciences. St. Petersburg. 1997. 20p. (russian)
- [10] L.Ya. Solovyeva, I.V. Churilova, V.A. Kniazhev, V.G. Kaloshin, T.O. Antipova, N.M. Fedorova. Method for the isolation of superoxide dismutase. The patent of the Russian Federation. No. 2186848. 10.08.2002.
- [11] Eremin A.N., Metelitsa D.I. The use of reversed Aerosol OT micelles in heptane for the purification of superoxide oxidase from blood erythrocytes. Prikl. biochem. and microbiol. (RU). 1996. Vol32. No.3. P.284-289. (russian)
- [12] J.A. Baum, J.G. Scandalios. Isolation and characterization of the cytosolic and mitochondrial superoxide dismutase of maise. Arch. Biochem. Biophys. 1984. Vol.206. No.2. P.249-264.
- [13] J. Kwiatowsky, A. Safianowska, L. Kaaninga. Isolation and characterization of an iron-containing superoxide dismutase from tomate leaves Lypopersicon exulentum. Eur. J. Biochem. 1985. Vol.146. No.2. P.439-466.
- [14] S. Reddy, K. Vijaya, H. Savithiri, B. Venkaiah. Isolation of isoenzymes of superoxide dismutase of isoenzymes of superoxide dismutase from bajra (Pennisetum typhoidam) seedlings. Biochem. Int. 1986. Vol.13. No.4. P.649-657.
- [15] O.I. Loseva, V.V. Osipov, E.N. Vanyakin, A.V. Gavryushkin. Allocation superoxide dismutase using the free stream electrophore sis method. *Biotechnology* (RU). 1995. No.5-6. P.25-29. (russian)
- [16] V.P. Komov, N.V. Kirillova, N.V. Alekseichuk. Isolation and purification of aldolase from tissue culture Rauwolfia serpentina Benth. Biochemistry (RU). 1986. Vol.51. No.7. P.1180-1185. (russian)
- [17] V.P. Komov, N.V. Kirillova, L.A. Troitskaya. Isolation and purification of peroxidase from callus culture of the Panax Ginseng LX-5 tissue. Applied Biochemistry and microbiology (RU). 1998. Vol.34. No.5. P.495-498. (russian)
- [18] N.S. Pivovarova, N.V. Kirillova, I.E. Kaukhova, L.I. Slepyan, and A.L. Marchenko. Development of water extraction technology on the basis of the biomass of polyscias Filicifolia (moore ex fournier) bailey strain. Butlerov Communications. 2016. Vol.45. No.2. P.113-118. ROI: jbc-02/16-45-2-113
- [19] J. Porath, J. Carlson, I. Olsson, G. Bilfruge. Metal chelate affinity chromatography a new approach to protein fractionation. Nature (London). 1975. Vol.258. P.598-604.
- [20] O.A. Keltsieva, V.D. Gladilovich, Ye.P. Podolskaya. Metall-affine chromatography: basis and application. Scientific instrument making (RU). 2013. Vol.23. No.1. P.74-85. (russian)
- [21] Pisetskaya N.F. On the selection of nutrient medium for ginseng tissue culture. *Plant resources.* **1970**. Vol.6. No.4. P.516-522. (russian)
- [22] Volosovich AG Culture of Rauwolfia tissue as a producer of antiarrhythmic alkaloids. *Author's abstract* of dis. doct. biol. sciences. St. Petersburg. 1992. 38p. (russian)
- [23] Y.P. Misra, I. Fridovich. The univalent reduction of oxygen by reduced flevins and quinines. J. Biol. Chem. 1972. Vol.247. No1. P.188-192.
- [24] O.H. Lowry, J. Rosenhrough, A. Farr, R.J. Randall. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951. Vol.193. No.1. P.268-275.
- [25] C. Beauchamp, I. Fridovich. Isoenzymes of SOD from wheat germ. *Bichem. Biophys. Acta.* 1973. Vol.317. No.1. P.50-64.
- [26] F. Paoletti, D. Aldimecci, A. Mocali, A. Caparrini. Sensitive spectrophotometric method of determination of SOD activity in tissue extracts. Anal. Biochem. 1986. Vol.154. No.2. P.536-541
- [27] B.L. Geller, D.K. Winge. A method for distinguishing Cu,Zn- and Mn-containing SOD. Anal. Biochem. 1983. Vol.128. No.1. P.86-92.
- [28] J. Porath, B. Olin. Immobised metalion affinity adsorbtion and immobilised metal ion affinity chromatography of biomaterials serum protein affinities gel-immobilised ion and nickel ions. Biochemistry. 1983. Vol.22. No.7. P.1621-1630.