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# Research Advances in Succinate Dehydrogenase

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## Abstract

**Succinate dehydrogenase is a mitochondrial marker enzyme. It is one of the hub linking oxidative phosphorylation and electron transport. It can provide a variety of electron in respiratory chain for eukaryotic and prokaryotic cell mitochondria. Its activity is generally used as indicators of the degree evaluation of the citric acid cycle run. This article summarizes the separation, active investigation, and the structure and nature of recent succinate dehydrogenase succinate dehydrogenase, succinate dehydrogenase applications. It aims to provide a reference for related research about succinate dehydrogenase.**

***Keywords: Succinate dehydrogenase; Activity assay; Properties; Application***

## Introduction

Succinate dehydrogenase (Succinate dehydrogenase, also called SDH) is a flavin enzymes enzyme. It is the inner mitochondrial membrane-bound enzyme, a membrane-bound enzyme. It is one of the hub linking oxidative phosphorylation and electron transfer, can provide a variety of electron in respiratory chain for eukaryotic and prokaryotic cell mitochondria, it is a sign of mitochondrial enzymes. As a key enzyme involved in the citric acid cycle, it is a reflection of succinate dehydrogenase mitochondrial function marker enzyme (marker enzyme) one. Its activity is generally run as an evaluation index of the degree citric acid cycle [1]. The research for it may have important significance for the evaluation of sperm mitochondrial function, researcher's induced pathological process Cow-one deficiency [2,3].

## The structure and pro perties of succinate dehydrogenase

**The structure of succinate dehydrogenase:** Succinate dehydrogenase is the citric acid cycle and aerobic respiration functional ingredients. It can be connected in series in the mitochondrial matrix (matrix or bacteria) to the oxidation of succinic acid and fumaric acid, ubiquinone reduction in film. Many bacteria and mammalian mitochondrial succinate dehydrogenase subunit is composed of two hydrophilic, namely flavoprotein subunit and iron-sulfur protein subunit. As well as two hydrophobic subunits, which are membrane anchored subunit contains heme b, to provide ubiquinone binding site. In eukaryotes, nuclear genes encoding mitochondrial SQRs gene mutations, showing a wide variety of clinical disease phenotype, including vision atrophy, tumor formation, myopathy, encephalopathy. SQR gene mutations include two kinds: (1) SdhA mutations behave like the TCA cycle gene defects, including Leigh's syndrome; (2) SdhB, SdhC, SdhD mutations cause hereditary paraganglioma tumor or pheochromocytoma appears. In the nematode mev-1 mutants, there is a mutation site on SdhC small subunit, found very sensitive to oxygen, can occur premature aging phenotype. Although some people

 

think that these disorders is complex Ⅱ oxidative stress caused by their own, but the molecular biological mechanism has not been elucidated.

Water-soluble succinate dehydrogenase containing non-heme iron metal isoalloxazine protein, highly purified enzyme preparations amber within a range not exhibit fluorescence Ph3-7 can crystallize crystalline trypsin and chymotrypsin processing purified water-soluble succinate dehydrogenase, after extraction of cresol, mercury sulfate precipitation, decomposition of hydrogen sulfide and paper electrophoresis, paper chromatography and other purification methods can be four kinds of adenine with a different peptide chain isoalloxazine nuclear nucleotide. From the analysis of their constituent and observe their nature, many researchers believe that they are known isoalloxazine adenine dinucleotide slightly different. Peptide chain attached isoalloxazine, its connection with the general isoalloxazine different proteins. The results of amino acid composition of the peptide chain part, to prove that they contain cysteine.

Iron non-heme iron, the iron content of the enzyme succinate dehydrogenase enzyme with increasing bluntness increases, succinate dehydrogenase in the presence or absence of iron regardless of the substrate are in a reduced state. After enzyme solution was losing its vitality oxygen in the air, where the iron does not change. Without the presence of substrate, enzyme solution and hydrogen acceptor (such as potassium cyanide or ferric nitrous anthracene carboxylic acid) wherein the continuous insulation not make the iron oxide. When the enzyme solution and phenanthroline insulation, part of the iron can be dialyzed out, the loss of enzyme activity and loss of iron content are closely related, but $Fe^{2+}$ or $Fe^{3+}$ did not make the enzyme activity recovery. In addition to iron dialysis, the partial protein denaturation, but in general dialysis skin conditions, protein invariance, nor the loss of vitality, may be combined with the iron of the enzyme prion protein to maintain a certain configuration, but after removal of iron protein gradual degeneration, it no longer allows the recovery of enzyme activity. Iron is one of the succinate dehydrogenase compositions. Iron and protein enzyme closely, it is closely related enzyme activity [4].

Inorganic phosphorus can increase the vitality of succinate dehydrogenase, succinate dehydrogenase, but not necessary to rely on the vitality of inorganic phosphorus in the presence of EDTA and inorganic phosphorus alanine has a similar effect. Inorganic phosphorus, ethylene diamine tetra acetic acid and phenylalanine may remove some of the increase in enzyme activity was inhibited leaving the vitality of heavy metals.

## The properties of succinate dehydrogenase

**Stability:** Water-soluble succinate dehydrogenase in the presence of oxygen in the air, the very unstable even if stored at -15°C and the bottom material is present. For enzyme failure in the air, was added ethylene diamine tetra-acetic acid cannot be prevented; cane sugar, glycerin or glycine is a considerable protection. However, in vacuum or oxygen-free nitrogen it is very stable. In the vacuum, 0°C to save two months have not found their activity decreased. Loss of vitality in the air enzyme preparations cannot be the use of $H_2S$, BAL or reduced glutathione (glutathione), in the absence of oxygen by thermal methods to reactivate it, view of these facts, and loss of vitality in the air From time to time simply due to oxidation caused by -SH.

**Activation of bottom material:** Particle binding of succinate dehydrogenase and succinate dehydrogenase can be due to a water-soluble base material and insulation base material is activated. In general, often enable more than doubled. Highly purified enzyme preparation is also preserved in this feature. The enzyme is activated when the acid was removed by vacuum dialysis lose some vitality, but still can be re-activated acid.

**Role of succinic acid concentration on the vitality:** Succinic acid at a concentration of $2 \times 10^{-2}$, the succinate dehydrogenase vitality nearing saturation. The method according to Lineweaver and Burk [4] calculated Michaelis constant at 0°C and 38°C and 1.9 each 0.58 mM, the latter with Thorn [5] myocardial formulation is similar to the determination of the material obtained data very consistent.

**Effect of different receptors on the enzyme activity:** When positive ferricyanide as receptors, receptor concentration increases, the dynamic gradually increased until the concentration exceeds 5 mM inhibited the enzyme activity. When 5 mM or less in a straight line reciprocal concentration reciprocal and dynamic relationship between the concentration of ferric cyanide.

**Influence of pH on the enzyme activity:** Effect of pH on the water-soluble succinate dehydrogenase in phosphate buffer is optimum pH 7.8.

**Effect of temperature on succinate dehydrogenase activity:** The effect of temperature on the viability of the succinate dehydrogenase shows the relationship between reaction rate and temperature according to the Arrhenius approach. The activation energy holding variable only within a very narrow temperature range, the higher the temperature, the lower the activation energy. Enzyme activation primer before the same activation energy.

**Extraction and physiological significance of succinate dehydrogenase:** Succinate dehydrogenase present in all cells of aerobic respiration, and mitochondrial membrane firmly bonded, is the only Krebs cycle and endometrial bound enzyme, is the most important dehydrogenase enzyme. So far, the purified enzyme from a variety of prokaryotic and eukaryotic tissues isolated for enzymology enzyme foundation. As a membrane enzyme succinate dehydrogenase mitochondrial membrane lipid bilayer structure having a combination of relatively tight, difficult to dissolve down, and leave it once the film is exposed to the air will soon be deactivated, so it's difficult to purify great. 1950 conducted a famous biochemist Lai [6], research on membrane proteins succinate dehydrogenase, after repeated experiments last butanol extraction method successfully succinate dehydrogenase from rat liver mitochondrial membrane dissolve down to give a high-purity water-soluble succinate dehydrogenase, its vitality than those reported by 1 time higher than the same period, which laid the foundation of leadership [6] in the field of succinate dehydrogenase. Thereafter, Davis et al. [7,8] using $NaClO_4$ this enzyme from bovine heart mitochondria dissolved Hattori and Asahi [9] selected ionic detergent sodium deoxycholate extracted from sweet potato root mitochondrial. Zheng [10] for *P. falciparum* was purified succinate dehydrogenase Xia et al. [11] etiolated corn seedlings biological material to obtain mitochondria by differential centrifugation, the use of ultrasound its broken, with 2% Triton X-100 soluble membrane, ultracentrifugation, ammonium sulfate precipitation, DEAE-C32 chromatography succinate dehydrogenase Xin and Zhou [12] by conventional enzymatic methods from psychrophilic yeast in (Y18) separated and purified succinic acid dehydrogenase catalytic activity.

Succinate dehydrogenase is directly connected to the electron transport chain, hydrogen acceptor is FAD rather than $NAD^+$, which allows the oxidation of FADH2 succinic acid and fumaric enzyme produced during the combination of the two electrons is transmitted directly from FADH2 enzymes to $Fe^{3+}$. Existing studies show that succinate dehydrogenase by the α, β two subunits, α subunit relative molecular mass of $70.0 \times 10^3$, containing FAD and two iron-sulfur cluster; β subunit relative molecular mass of $27.0 \times 10^3$ containing one iron-sulfur cluster.

**Common measurement method for succinate dehydrogenase**

**Phenazine methosulfate (PMS) reaction** [13,14]: Succinate dehydrogenase through a series of artificial electron acceptors, such as the PMS (phenazine dimethyl sulfate), DCPIP catalytic oxidation of succinic acid (2,6 Dichlorophenolindophenol), etc. occur, and by means of color change these intermediates can be quantified by spectrophotometer reflect, the reaction is:

①Succinate+PMS→Fumarate+PMSH2;

②PMSH2+DCPIP→PMS+DCPIPH2

DCPIP blue, standard absorption spectrum at 600 nm, the color gradually fades to its reduction, which is proportional to the change in optical density at 600 nm with DCPIP content was measured reduction rate 2.6-DPIP can calculate SDH vitality. DCPIP molecule is reduced, which represents one molecule of succinic acid oxidation. It can be determined that this reaction system to absorb changes in brightness at 600 nm in to calculate SDH activity. SDH activity was calculated:

(Standard - Determination) / Standard = μmol/min/mg

**Potassium ferricyanide [$K_3Fe(CN)_6$] reduction:** Potassium ferricyanide [$K_3Fe(CN)_6$] and sodium succinate as substrate, so that succinate dehydrogenase catalyzed reaction potassium ferricyanide is reduced to potassium ferrocyanide [$K_4Fe(CN)_6$], $K_4Fe(CN)_6$ and then with $Fe^{3+}$ and generated Prussian blue, in the 700 nm wavelength measured absorbance values to detect generation amount of Prussian blue, succinate dehydrogenase as reducing power, the higher the absorbance value indicates succinate dehydrogenase stronger.

**TTC (Triphenyltetrazolium chloride) method:** Colorless TTC (2,3,5- triphenyltetrazolium chloride) as artificial by hydrogen, which accepts hydrogen in cellular respiration process, reduced to triphenylsilyl meal (TF). The latter form of red crystals is present in the cell, using an organic solvent (e.g., toluene, ethyl acetate, chloroform, acetone, ethanol, or the like) for extraction. The extract was 485 nm absorbance was measured after the reduction amounts to TTC represents dehydrogenase activity was calculated according to the amount of TF generate a standard curve, and then find TTC-dehydrogenase activity.

**MTT method:** MTT is a yellow dye. Mitochondrial succinate dehydrogenase in living cells can restore the metabolism of MTT, while generating a blue (or blue-violet) under the action of cytochrome C of water-insoluble formazan (Formazana), the role of particles dissolved by isopropanol color. Under normal circumstances, the amount of formazan generated is proportional to the number of living cells, so the viable cell number can be presumed according to the optical density OD value at 570 nm.

MTT assay test analysis method of high background, low sensitivity, poor stability factors, improves the experimental conditions to improve sensitivity and stability, reduce background. With MTT colorimetric method, cytotoxicity assay and

cell proliferation assays, analytical performance characteristics under different conditions were compared MTT chromogenic reaction. Comparison of the 13 kinds of solvent solubility of Formazan. Analysis examined the succinate dehydrogenase in the cell distribution, pH value on the MTT reaction system, phenol red and impact bovine serum test results, the corresponding countermeasures. Detecting the response time curve and MTT cell viability curve. Analysis of the sensitivity and stability of the improved MTT assay method. Conclusion: MTT as an enzyme substrate in cellular metabolism mainly on the cell surface. pH MTT reaction system is 6 • 7, good Formazan solvent is n-propanol, isopropanol and ethylene glycol ether. Remove the cell culture supernatant, detect enzyme cells, denature proteins with a solvent, to remove the precipitated proteins influence, MTT assay method enables to reduce the background, sensitivity increase. Typically the method detects K-562 cells and human lymphocytes sensitivity of about 1000 and 10,000 cells respectively.

**NBT (Nitroblue tetrazolium) method:** NBT soluble in water, pale yellow, with NBT as by hydrogen, sodium succinate is accepted by the action of the enzyme off the hydrogen, thus the formation of purple blue precipitate PMS was added in the reaction system, mixing, 37℃ incubated 30 min, the reaction was terminated after addition of TCA. Isopropyl alcohol to dissolve the color, mix, 548 nm OD value can be measured in. It states: within 30 min, 548 nm OD value is the amount of enzyme under 1.0 is a dynamic unit. Specific activity = Activity unit / mg protein [15].

**Improved detection of succinate dehydrogenase**

As a marker enzyme reaction in the group commonly long shot dehydrogenase to reflect three shuttle acid cycles. In the study of bone and bone cell growth and metabolism in order to beat Rose dehydrogenase activity was observed indicators that make sense. Because bone tissue contains large amounts of inorganic salts and calcium, very hard, in the study require decalcification, and in the group of the reaction biggest difficulty is decalcification solution can reduce activity, bone decalcification by enzyme activity is affected. Therefore, the author first decalcification after incubation method applied under the collar bone graft and implant sell experiments to solve the bone demineralization activity in question [16].

**The activity change of cell succinate dehydrogenase in different training load:** Sugar aerobic oxidation system in motion with energy consumption and occupies a pivotal position, succinate dehydrogenase is a functional component of the citric acid cycle and aerobic respiration is one of the hub linking oxidative phosphorylation and electron transfer, respiratory chain can provide a variety of eukaryotic and prokaryotic cell mitochondria and aerobic capacity of electron, as a sign of mitochondrial enzymes. Many studies have shown that skeletal muscle succinate dehydrogenase activity is closely correlated with the intensity of training. Experiment, 4 and 6 weeks of aerobic exercise group and aerobic anaerobic alternating movement 2 weeks of skeletal muscle succinate dehydrogenase activity increased activity of other groups no significant change. It showed that 4 to 6 weeks of aerobic treadmill training rats can receive appropriate stimulation, skeletal muscle to produce adaptive changes in skeletal muscle succinate dehydrogenase activity increased. After Huertas et al. [17] of 14 long distance runners embodiment four weeks roadwork biopsy found he walks gastrocnemius muscle succinate dehydrogenase activity was significantly increased, indicating that the appropriate exercise training can make muscle aerobic oxidase produce adaptive response. Fournier et al. [18] found that six or seven men, after three months of endurance training significantly increased skeletal muscle succinate dehydrogenase activity, whereas anaerobic training men after 8 weeks sprint training succinate dehydrogenase activity unchanged. Costill et al. [19] engaged in track and field athletes muscle succinate dehydrogenase activity, and found that the highest Distance Runners succinate dehydrogenase activity, slight increase

sprinters, and succinate dehydrogenase activity and field athletes and ordinary people no difference. In addition, the experiment also found that a short period of alternating aerobic anaerobic training is also conducive to the protection of the enzyme activity, improve the ability of aerobic oxidation of energy supply, but be careful to take the training time and intensity.

**Application of succinate dehydrogenase:** SDHIs class (succinate dehydrogenase inhibitors) of fungicides is a mechanism of action and resistance mechanism is Fungicide Resistance Action Committee (Fungicide Resistance Action Committee, FRAC) new division out similar compounds. The mechanism of inhibition of these fungicides is mainly pathogens succinate dehydrogenase activity, thus interfering with respiration [20]. 1960s developed carboxin (carboxin) is the earliest varieties of such fungicides have been applied 40 years, followed by the development of oxycarboxin (oxycarboxin). But these species control spectrum is narrow, it can only be used for prevention and treatment limited types of diseases, such as chrysanthemum genus rust (*Puccinia oriana*) and barley loose smut (*Ustilago nuda*) and the like. With the development of in-depth research and technology create new compounds, more broad-spectrum fungicide SDHIs have been successfully developed, such as mepronil (mepronil), flutolanil (flutolanil), wheat rust Ling (benodanil), furosemide amide (fenfuram), boscalid (boscalid), ceftiofur amide (thifluzamide), furametpyr (furametpyr), topiramate thiophene amide (penthiopyrad) and nearly two years of research and development of new bacteria flurbiprofen amide, and they can be used prevention and treatment of many diseases of various crops. The production has become a very important class of microbicides varieties. But with the widespread use of such agents, due to a single site, it is also becoming increasingly prominent problem of resistance [21] it is one of the hot fungicides in this research.

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