

Many faces of superoxide dismutase, originally known as erythrocuprein

T. Ramasarma

Superoxide dismutase (SOD), discovered in 1969 by Fridovich, was found to be identical with the ubiquitous copper protein, erythrocuprein, originally described by Keilin. SOD had great influence in the studies on oxygen radicals and toxicity. New reactions, now identified with this protein, expand its potential beyond dismutation of superoxide.

By virtue of being a copper protein with accessible metal centre, the SOD protein shows efficient reversible electron transfer with superoxide ($O_2^{\bullet-}$). So do others in addition to $O_2^{\bullet-}$. Utilizing this potential, SOD can nullify autoxidation by dismutating the two radical products formed in the first step of electron transfer. This brings out a true antioxidant function of SOD of saving catechol, thiol and other compounds from autoxidation loss.

Among the emerging novel functions, two examples are outstanding. First is the protection against autoxidative inactivation of calcineurin (a protein phosphatase) by the native SOD protein, but not its mutant forms. Second is the ability of exogenously added native SOD as well as metal-free protein (with no possibility of dismutation activity) to regulate an intracellular enzyme, HMGCoA reductase, and thereby cholesterol biogenesis.

A quantum jump has been made in understanding the versatility of the SOD protein in cell functions by the discovery of mutants in the neurodegenerative disease, familial amyotrophic lateral sclerosis. These mutant proteins retain the dismutase activity to varying degree (like isoenzymes), have changes in structure and folding and an increased tendency to form aggregates and insoluble complexes, and assume activities that are toxic.

Keywords: Calcineurin inactivation, cholesterol biogenesis, pyrogallol autoxidation, superoxide dismutase.

‘Disproving an accepted theory is the only way to advance knowledge.’

—Karl Popper

AN enzyme is no longer specific for one action as we were led to believe. The number given to it in the enzyme commission nomenclature refers to the activity and not the protein. The phenomenon of ‘one protein – many activities’¹ appears to be a cellular design for multiplying catalytic potential of the limited genes in action. Mutants of a protein gain in new functions and thus add on more actions. Search for these is now limited to diseases such as cancer and multiple sclerosis. It is equally possible that a cell uses some of these functions of a protein. This article explores this phenomenon using superoxide dismutase (SOD) as an example. A famous name and an unquestioned belief that enzymes are specific had indeed stifled

thinking of alternatives. Hopefully this perspective will throw open windows for fresh air to enter.

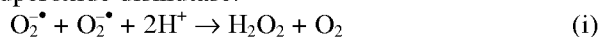
Dismutation of superoxide, the first enzyme activity of ubiquitous copper protein, erythrocuprein

A blue copper protein was isolated in 1938 by Mann and Keilin² from erythrocytes and liver. It was found to be ubiquitous in animal tissues, prompting the names hemocuprein, erythrocuprein, cerbocuprein, hepatocuprein and cytocuprein, all of them were found to be identical, and cytocuprein was recommended as the name for this ubiquitous cellular copper protein³. Its function was unknown. It is normal that when a catalytic activity is found in a tissue extract, the protein responsible is purified by classical fractionation methods and characterized. On the other hand, when a protein is available it is difficult to predict its activity. This protein shot into prominence after discovery of its catalytic activity of dismutation of superoxide. It is instructive to read the personal account of

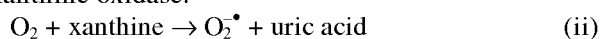
T. Ramasarma is in the Centre for DNA Fingerprinting and Diagnostics, Hyderabad 500 076, India and Solid State and Structural Chemistry Unit and Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India. e-mail: ramasarma@hotmail.com

Fridovich⁴ on this discovery of an enzyme with activity of dismutation of two molecules of superoxide ($O_2^{\bullet-}$) to H_2O_2 and O_2 (reaction i). It began with the observation that inhibition of reduction of cytochrome *c* in xanthine oxidase reaction by a tissue extract depended on oxygen. And the rest was history. The purified protein, a dimer with molecular weight of 32,000 with one copper and one zinc atom per subunit, was found to be identical with erythrocuprein. Fridovich and coworkers^{5,6} brilliantly uncovered the story: $O_2^{\bullet-}$ produced on oxidation of xanthine by xanthine oxidase (XO) (reaction ii) reduced cytochrome *c* (reaction iii), and by its removal the tissue extract inhibited reduction of cytochrome *c*. It was deduced that an enzyme in the tissue extract removed $O_2^{\bullet-}$ by catalytic dismutation of the radical.

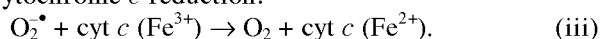
Superoxide dismutase:



Xanthine oxidase:



Cytochrome *c* reduction:



The protein was rechristened superoxide dismutase (SOD) by its enzyme reaction. It had a glorious run in illuminating the importance of oxygen radicals and oxygen toxicity. SOD became an icon and its veneration led to the dogma that any reaction inhibited by SOD must find an explanation based on, and only on, superoxide and its dismutation. Thus the overpowering name, SOD, did some disservice in preventing thinking of its alternative actions. In the context of the concept of 'one protein – many functions' now applicable to many proteins⁷, it is time to accept that the ubiquitous, abundant protein SOD will have other functions beyond its name. Indeed many such are forthcoming in the last decade. Some of these are described here.

Inhibition of autoxidation of catechol compounds

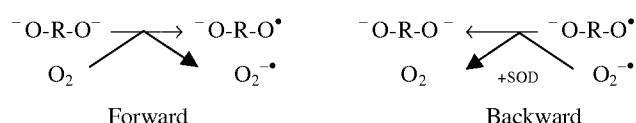
In the first major paper on identification of enzyme activity, McCord and Fridovich⁵ had recorded that SOD protein also inhibited autoxidation of a catechol compound adrenaline to adrenochrome. The reaction mixture contained xanthine oxidase + xanthine that produced superoxide. This led Misra and Fridovich⁶ to interpret the reaction as 'cooxidation' of the catechol by superoxide, removal of which by SOD showed up as inhibition. Similar inhibition by SOD of autoxidation at alkaline pH of other catechol compounds 6-hydroxydopamine⁸ and pyrogallol⁹ was reported. Amazingly, superoxide involvement was unquestioningly accepted in the face of the glaring fact that these autoxidations tested at alkaline pH, occurred at high rates without external superoxide.

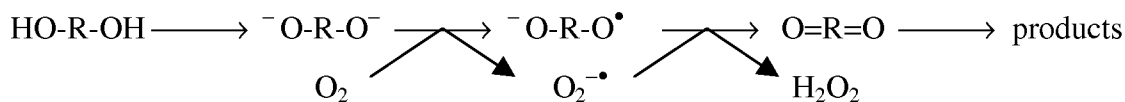
Pyrogallol is rapidly oxidized accompanied by oxygen consumption in alkaline buffers of pH 8.0 and above with the colour of the solution changing from brown to green to yellow. This reaction is studied in presence of EDTA to eliminate the part due to contaminating metals. Addition of excess catalase released back into the medium up to 50% of consumed oxygen, indicating presence of H_2O_2 as the product. The autoxidation reaction can be represented as transfer of two electrons from oxy-anions to oxygen in two steps. The first reaction represents ionization of the ortho-hydroxy oxygen atoms by alkali, and the last reaction the conversion of quinone to other products (Scheme 1).

The most widely used assay method introduced in 1974 by Marklund and Marklund⁹ involves concentration-dependent inhibition by SOD of autoxidation of pyrogallol. Progress of the reaction can be followed either by oxygen consumption or by formation of the product, purpurogallin (λ_{\max} 420 nm). The reaction continued at a decreased rate in the presence of SOD. Extrapolating such data for the amount of protein required for 50% inhibition at a fixed pyrogallol (substrate) concentration formed the basis of quantitation of SOD. This concentration of SOD required for 50% inhibition is known to increase with increased pH or pyrogallol concentration. And these conditions also increased the rate of autoxidation reaction. Thus comparison of activities must strictly adhere to fixed pyrogallol concentration and pH. Despite enormous interest in SOD assay, no assay method based on rate of disappearance of the substrate or of formation of the product, usual for enzyme assays, is available.

Attempts made to explain this inhibition relied on the well-known dismutation function of SOD. Notice from the reaction sequence in Scheme 1, that removal of $O_2^{\bullet-}$ by dismutation to $\frac{1}{2}H_2O_2 + \frac{1}{2}O_2$ (reaction i) by SOD should always produce 50% decrease in the rate of oxygen consumption (ΔO_2 , $\mu M/min$). But experimentally inhibition is proportional to concentration of SOD, and excess SOD nearly abolishes autoxidation. To fit with the theory, superoxide from another source such as xanthine oxidase system⁶ was provided and assumed to be the primary oxidant. It is well known that autoxidation of pyrogallol in alkaline medium requires no external source of superoxide. Thus inhibition of catechol autoxidation by SOD is beyond doubt but not so the explanation of its action.

Notice that the two products of the forward reaction in Scheme 1 are oxygen radicals. SOD knows how to handle them. Then it becomes self-evident that by catalysing dismutation of these radicals, $^{\bullet}O-R-O^{\bullet}$ and $O_2^{\bullet-}$, SOD protein can drive the reaction back and nullify autoxidation.





Scheme 1. Representation of two one-electron transfer steps during autooxidation of a catechol by oxygen.

Our finding that the action of added SOD is limited to constant decrease in activity (inhibition) at two high substrate (pyrogallol) concentrations irrespective of varying activity of autooxidation¹⁰ provided crucial evidence for this. We interpreted that SOD achieves this by reversing the first step of electron transfer, saving the substrates, phenolate and oxygen. Then decrease in the rate of autooxidation represents the rate of SOD-catalysed back reaction. This interpretation of SOD activity and a simple modification of the assay by measuring inhibition of autooxidation at two high concentrations of pyrogallol can give a rate measurement of its catalysis¹⁰, like all other enzymes.

The rate of autooxidation of pyrogallol at pH 7.5 is about one-quarter of that at pH 8.0 and saving by SOD from loss under physiological conditions will not be insignificant. Similar results were obtained on the effect of SOD on autooxidation of noradrenaline, dopamine and polyphenols of tea extract. By this action SOD can prolong the life and action of catechol compounds, such as endogenous neurotransmitters and exogenous antioxidant compounds, a valuable addition to the biological activities of SOD. By saving autoxidizable compounds, SOD becomes a true antioxidant.

Vanadate-dependent oxidation of NADH – a case of SOD action on non-oxygen radicals

In 1981, Crane and co-workers discovered inhibition by SOD of vanadate-dependent oxidation of NADH by O₂ catalysed by membrane enzymes^{11,12}. Many laboratories confirmed this crucial finding. How does one explain the paradox of inhibition of generation of H₂O₂ (thought at that time as the product) by SOD, the enzyme that forms H₂O₂ by dismutation of superoxide? SOD should have helped in speeding up the process instead.

In 1984, Darr and Fridovich¹³ reported that vanadate stimulated 'the oxidation of NADH by superoxide radical' using xanthine oxidase + xanthine system, purportedly as a source of superoxide, and this was inhibited by SOD. Based on the surmise that SOD inhibition must mean participation of superoxide, they proposed a bizarre entity V^{IV}-OO (notice valency of terminal oxygen atom?) as the oxidant, formed from V^V and O₂^{•−} (electron transfer gives V^{IV} + O₂; addition compound will be V^V-OO[•]). We found that the membrane-derived xanthine oxidase protein by itself catalysed the reaction in the absence of xanthine, and therefore without superoxide¹⁴. Shut out by the unshakable belief that SOD can only dismutate superoxide,

explanation of this SOD inhibition remained vague. Any proposal of these reactions and effects must accommodate participating vanadate forms.

Given below are some characteristics of this unusual NADH oxidation reaction, defined by the work from our and other laboratories, and summarized in a review¹⁵.

1. NADH was oxidized accompanied by oxygen consumption (NADH : O₂ = 1 : 1) in the presence of polyvanadate (V₁₀ with traces of V₄, V₁), and not metavanadate alone.

2. Adding SOD stunned the reaction saving both NADH and O₂, nullifying the oxidation as it were.

3. Activity increased monotonously as the pH decreased.

4. Maximum activity depended on the combined presence of phosphate and metavanadate, and catalytic amounts of V₁₀ and diperoxovanadate (DPV).

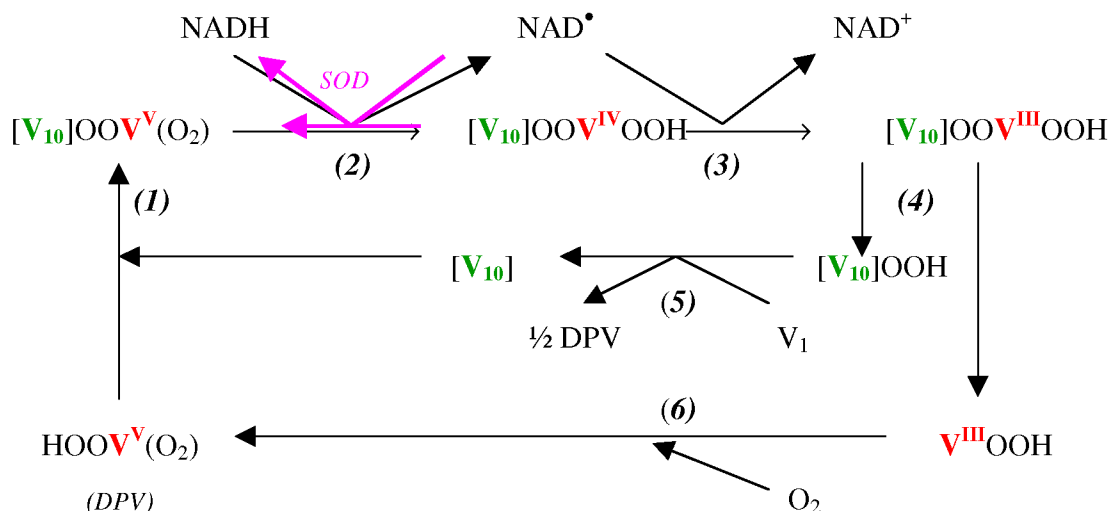
5. Half of consumed O₂ was released on addition of catalase but at a rate much slower than H₂O₂, indicating the presence of an altered form of peroxide. DPV was found to be the product, confirmed by its characteristic chemical shift in the ⁵¹V-NMR spectrum.

6. Decameric vanadate (V₁₀) is the preferred form for reduction. Its reduced form, [V₁₀]-V^{IV}, gives typical eight-banded ESR-spectrum of vanadyl radical.

This NADH oxidation system consumes NADH and O₂, produces ½DPV as the product (using free vanadate) and needs DPV and V₁₀ in traces, implying a cyclic operation. The active oxidant species is likely to be the peroxo-bridged adduct, known to form between [V₁₀]-V^{IV} and DPV-V^V. Phosphate probably stabilizes this complex. Scheme 2 fits all these observations in two cycles to regenerate [V₁₀]-V^{IV} and DPV-V^V. Notice the first electron transfer reaction is between NADH and V^V of the divanadate complex and produces NAD[•] and V^{IV}, both radical species, as in the case of pyrogallol autooxidation. It then becomes self-evident that SOD can dismutate these, nullify the forward oxidation, and decrease the rates of loss of NADH and O₂ (see also ref. 16).

SOD protects calcineurin from aerobic inactivation – another case of dismutation of radical products?

Calcineurin, a calcium-activated serine/threonine protein phosphatase (PP2B), is involved in many cellular processes such as regulation in the release of neurotransmitters and hormones, ion-channels and neuronal excitability, cell cycle, T-cell activation and apoptosis¹⁷. Calcineurin,



Scheme 2. Proposed scheme of vanadate-dependent oxidation of NADH. (1) Adduct formation between [V₁₀] and DPV. (2) First electron transfer from NADH to peroxo-bridged V^V complex. (3) Second electron transfer to V^{IV} complex. (4) Breakaway of V^{III}OOH on reduction to this lower valency state of V. (5) Sequestering the peroxide as DPV, the most stable form at pH 7.0, and releasing [V₁₀]. (6) Autoxidation of V^{III}, consumes O₂ forming bidentate-(O₂) of DPV, supported by experiments with salen-V^{III} of Liu and Anson¹⁶. Peroxy-vanadate cycle explains the catalytic role of [V₁₀] (green) (reactions 1–5) and of DPV (red) (reactions 1–4, 6). By dismutating the two radical products of reaction (2), SOD nullifies the first step of electron transfer (crimson).

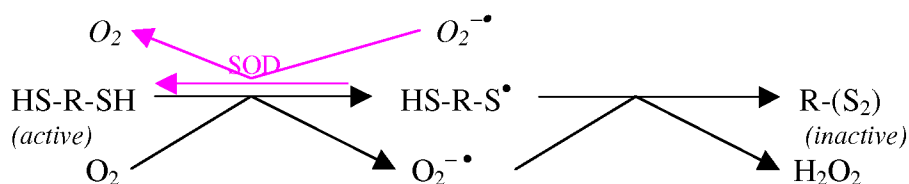
a heterodimer of catalytic subunit (60 kDa) and regulatory subunit (19 kDa), is enriched in neural tissue to the extent of 1% of its protein¹⁸. The active site in the catalytic subunit has a binuclear centre with Fe²⁺.Zn²⁺, and possibly three redox-active cysteine residues. On treating with Ca²⁺-calmodulin, the protein ‘opens’ and becomes active on treatment with ascorbate and dithiol agents. The protein also requires Ca²⁺-calmodulin for inactivation on exposure to air¹⁹, superoxide²⁰, and H₂O₂ (ref. 21). Only inactivation by air and by superoxide, but not by H₂O₂, was prevented by SOD. As usual with such findings, superoxide-dependent oxidative inactivation is proposed primarily targeting Fe³⁺ of the bimetal centre, and in some cases dithiol oxidation to disulphide. Involvement of two, perhaps three, thiol groups, and oxidation of Fe²⁺ of the metal centre in the overall inactivation was indicated by reactivation by DTT, ascorbate and Fe²⁺. Inactivation by H₂O₂ seems straightforward oxidation of dithiols to disulphide that can be reversed to a large extent by thiol agents. Oxidative inactivation by air (O₂), and its prevention by SOD, led to the expected presumption that superoxide is the oxidant. The target of action is less

clear. It is known that thiol groups in some proteins are prone to autoxidation in air (O₂). We propose that inactivation of calcineurin may follow the pattern of autoxidation described above. It follows that prevention of this by SOD by reversal of the first electron transfer step leads to protection of the enzyme as given below. A dismutation between S[•]-radical and superoxide in the backward reaction (crimson) is a logical action of SOD (Scheme 3).

A prediction arising out of this proposal is that the decrease in activity by a given concentration of SOD will remain constant at high concentrations of the substrate, calcineurin protein.

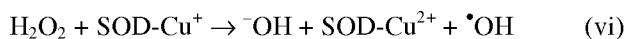
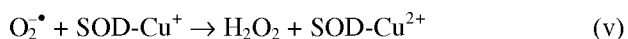
Reversal of SOD reaction with H₂O₂ as the electron donor

The forward dismutation reaction of SOD occurs in two successive steps of electron transfer wherein superoxide acts as both donor (reactions iv) and acceptor (reaction v). The SOD-Cu²⁺ and SOD-Cu⁺ couple facilitates this electron transfer. H₂O₂ thus formed (reaction i) is known to



Scheme 3. Possible action of SOD in reversing the first electron transfer step in autoxidation of calcineurin.

inactivate SOD itself, by generating $\cdot\text{OH}$ radicals on interacting with SOD-Cu^+ in a Fenton-type reaction (reaction vi).

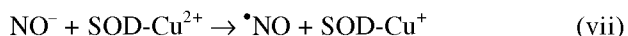


Generation of hydroxyl radicals by this peroxidative activity of SOD was confirmed by chemical and ESR studies with spin traps^{22,23}. There must be some way of protecting SOD from inactivation by its own product to which it is constantly exposed. A large number of small anions such as chloride and phosphate (and also cyanide, azide, vanadate tetramer) bind to the anion channel of this protein and protect it from self-inactivation. Another way is to transform H_2O_2 into a safe product, e.g. diperoxovanadate. This additive complex is stable at pH 7.0 and to action of catalase, substitutes H_2O_2 in peroxidative activities²⁴, and does not inactivate SOD. Chances of forming the destructive hydroxyl radicals by native SOD are meagre since millimolar concentrations of H_2O_2 used in these experiments are unphysiological. It is doubtful if this potential of SOD will ever be used.

Interaction with other radicals – SOD-Cu^{2+} can receive an electron from nitroxyl anion (NO^-) and catechol semiquinone

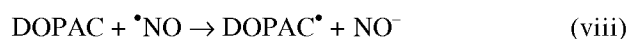
SOD has another interesting function of saving endothelium-derived relaxing factor (EDRF), identified as nitric oxide ($\cdot\text{NO}$). Explanation based on superoxide dismutation inexorably followed: SOD removes $\text{O}_2^{\cdot-}$ and saves $\cdot\text{NO}$ from converting to inactive peroxynitrite. A doubtful part in this explanation is the availability of sufficient superoxide in endothelial cells.

Studies on nitrogen radicals led to discovery by Murphy and Sies²⁵, of another activity of SOD. SOD-Cu^{2+} was found to oxidize nitroxyl anion (NO^-) to nitric oxide ($\cdot\text{NO}$). Thus the relaxing factor of smooth muscle will be available by a reaction other than nitric acid synthase, provided NO^- is available.



Autoxidation products of dopamine (quinone and semiquinone), as well as $\text{O}_2^{\cdot-}$ and H_2O_2 , induce neurotoxicity and loss of dopaminergic neurons in Parkinson's disease. The catechol groups of dopamine and its major metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), are autoxidized to their quinones²⁶. But for some reason, semiquinone of DOPAC (reaction viii), but not of DOPA, was formed on oxidation with $\cdot\text{NO}$, more readily than O_2 .

SOD enhanced this effect possibly by recycling the product, NO^- (reaction vii). These authors also showed that SOD enhanced by 2.5-fold the formation of DOPAC-quinone by dismutation of DOPAC \cdot (reaction ix), a disproportionation normally expected of such radical species.



It thus appears that SOD-Cu^+ can receive an electron from $\text{O}_2^{\cdot-}$, NO^- , or a catechol semiquinone, and possibly other radicals. Alternative actions of SOD should follow. Arising out of this realization some of the effects of SOD, limited to uncertain damage due to excessive superoxide, need reevaluation. One example is its protective effect in reperfusion/ischemic injury²⁷. It is not beyond the scope of SOD to be an alternative source of $\cdot\text{NO}$ and improve vascular tone, a significant factor in such injury.

SOD-deficient yeast survives well in high oxygen – whither oxidative stress?

Simple studies on the lifespan of yeast gave a clue for another action of SOD – a mutant of *Saccharomyces cerevisiae*, a facultative anaerobe that grows in aerobic and fully anoxic condition, with its SOD deleted. The wild type and the mutant showed similar replicative lifespan and the rate of cell division, regardless of concentration of O_2 (100, 21% or nil) to which they were exposed²⁸. The authors argued that the effect of SOD cannot be limited to removal of superoxide, and its participation 'in redox reactions other than dismutation of superoxide' is indicated. But they still retained redox reactions as an alternative. What their experiments showed clearly was that the SOD protein in the cell is required for its growth even in the absence of oxygen. A much-debated topic is the protection of cells exposed to so-called 'oxidative stress' by SOD by dismutating superoxide to H_2O_2 . This unchallenged perception disregards the fact that the presently accepted product of SOD, H_2O_2 , is a stronger oxidant than superoxide²⁹.

SOD protein alone is active in decreasing HMGCoA reductase and increasing protein kinase C

Mondola and coworkers³⁰ reported an unusual interaction of externally added SOD on the activity and concentration of an enzyme within the cell – a remarkable, path-breaking finding. A tiny amount of externally added SOD protein (3 nM, about 0.1 $\mu\text{g/ml}$) in the medium of hepatocarcinoma cells decreased incorporation of ^{14}C -acetate into cholesterol, and activity and concentration of the protein of HMGCoA reductase, the key regulatory enzyme in

cholesterol biogenesis. It is noted that the decreases were limited to about 50%. Most significantly, these effects were also obtained with metal-free and H_2O_2 -inactivated SOD protein. These effects are obviously independent of its dismutation activity. SOD in serum is found mainly with circulating low density lipoprotein (LDL)³¹ that services many tissues. Binding of LDL to the membrane, previously known to show these effects on cholesterol biogenesis, was indeed enhanced in the presence of SOD. However, the effects in hepatocarcinoma cells were observed in the absence of foetal calf serum, the source of LDL. Another possible explanation is redistribution of coenzyme Q (Q) to enrich microsomes, known to show such decrease in HMGCoA reductase limited to 50% on feeding exogenous Q or by increasing endogenous Q^{32,33}.

The SOD protein per se seems to be acting at the plasma membrane by binding to an undefined receptor and is known to be internalized by hepatic cells. Addition of SOD protein to hepatocarcinoma cells increased activity of cytosolic protein kinase C. These studies open the possibility of a broad impact of the SOD protein on cellular regulation independent of its metal content and dismutation activity. Fitting every action of SOD with dismutation of superoxide is counterproductive.

Multiple actions of mutant SOD proteins

Identification of modified SOD1 proteins in familial amyotrophic lateral sclerosis (FALS), a degenerative disease of motor neurons, spurred research that unfolded many alternative actions of the mutants of SOD. FALS is an age-related degenerative disorder of motor neurons of cortex, brainstem and spinal cord³⁴. Over 100 mutations of the gene SOD1 that codes for Cu, Zn-SOD are detected in about 25% of these cases³⁵. Loss of dismutation activity was suspected. It is so in some, but not all cases. Being uncertain and elusive, this was followed by a short-lived proposal of gain-in-toxic function such as production of dangerous hydroxyl radicals.

Presence of protein aggregates in neural cells is a characteristic of many neurodegenerative diseases. Most of current studies reveal that cytotoxicity might involve interactions of mutant proteins with many vital components. A summary is given of the unusual behaviour of some of the mutant SOD proteins (standard notation, e.g. A4V, alanine 4 changed to valine).

Motor neuron disease not correlated with SOD activity

Many of the mutants showed little change or small decrease in dismutation activity. It actually increased in G93A. The activity no doubt decreased in the active site mutant, H46R, but this mutant showed a mild form of FALS³⁶. In contrast, severe form of the disease was found in mutants

(A4V, G37R, G93A) with changes far away from the SOD active site. In the overall assessment of many investigators, the dismutation activity of SOD1 mutants has little to do with neural cell death in FALS³⁷.

Reverse reaction and toxic products

Formation of destructive hydroxyl radicals by SOD proteins in the presence of H_2O_2 was remarkably enhanced in the mutants A4V and G37R^{38,39}. Toxicity in FALS is proposed to be due to gain in this activity. In support of this, breaks in DNA, measured by decrease in supercoiling, were indeed observed in mutants A4V and G93A⁴⁰. This reaction needed unphysiologically high concentrations of H_2O_2 and therefore is of doubtful value.

High-molecular weight complexes of mutant SOD protein

Inclusion bodies of insoluble mutant SOD proteins were found in motor neuron cells of G93A and G85R⁴¹, a characteristic of the FALS disease. It is not known how these cause neurotoxicity.

Structural changes in mutant SOD proteins

Crystal structure of SOD in A4V mutant close to the dimer interface showed substantial reorientation of the dimers and destabilization of the protein⁴². Small molecules bind at the dimer interface and prevent aggregation of mutant SOD proteins of A4V, G93A, and G85R⁴³. Loss of Zn was also found in G85R and H46R. Coincidentally, partial unfolding of the SOD proteins of G93A, G85R, D90A and L38V was also observed⁴⁴. Such altered structure, correlated with decrease in Zn/Cu ratio, may be responsible for the decreased protection of protein phosphatase activity of calcineurin against inactivation on autooxidation by mutant proteins of A4V¹⁹, G93A^{19,45} and D90A⁴⁵.

SOD secretion impaired

Secretion of SOD, a disulphide protein, decreased with the mutant G93A⁴⁶. Functions outside the cell are already surfacing. Disorder around C57 and C146 that form the native intramolecular S-S bond leads to intermolecular S-S bond in some mutant proteins⁴⁷.

Some general remarks

The original Keilin's erythrocyte, more famously known as Fridovich's SOD, is expected to do more than mere dismutation of superoxide. The fast-accumulating information described here points to a much wider role, beyond redox,

for this protein. The number of examples of 'one protein – many functions'⁷ is growing. It is time to abandon the out-of-the-box approach of limiting the action of a protein to one reaction.

Other examples of metal-bound proteins with SOD activity are now found. Native prion protein (PRP^c) on reconstitution with copper showed superoxide dismutation activity⁴⁸. Germin, a manganese-containing protein, showed activities of oxalate oxidation and superoxide dismutation⁴⁹. Dismutation of superoxide is thus not exclusive to SOD protein. 'One function – many proteins' may also be found in other cases.

These days it is common in cell biology that several proteins, identified by their molecular weight (e.g. p53) or by three-letter code (e.g. *Ras*), await identification of their functions for which the cell makes them. In some cases the toxic action of a mutant in pathological state is known and the native functions are beginning to be deciphered (e.g. *Ras*)⁵⁰. Mutant forms of a protein can gain cytotoxicity is an emerging perspective. Formation of misfolded protein aggregates is a remarkable hallmark of various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease, prion encephalopathies and amyotrophic lateral sclerosis⁵¹.

Postulation of a free-radical sink is more appropriate for abundant tocopherol, ascorbate and glutathione rather than SOD. The carbon-centred free radicals, usually formed by metal-dependent oxidation, are attacked by readily available O₂ to form RCOO[•] radicals, which need to be reduced to RCOOH. Thus these oxidations, now understood in terms of stress, can be traced to hydrogen peroxide or carbon hydroperoxides and are more likely to be regulatory in nature. On the other hand, producing small concentrations of H₂O₂ needed for essential peroxidative reactions might indeed be a vital cellular role of SOD⁵².

SOD is often referred to as an antioxidant enzyme. The action of SOD in dismutating radical products of autoxidation and driving the reaction backward, according to our interpretation, saves autoxidizable components, including some thiol proteins. Thus it suppresses oxidation and is therefore a truly antioxidant enzyme.

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