Biochimica et Biophysica Acta, 526 (1978) 644—647

BBA Report

BBA 61344

INVOLVEMENT OF SUPEROXIDE IN THE CATALYTIC CYCLE OF DIAMINE OXIDASE

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(Received May 24th, 1978)

Summary

The reaction of pig kidney diamine oxidase (amine:oxygen oxidoreductase (deaminating) (pyridoxal-containing), EC 1.4.3.6) could be significantly inhibited by superoxide dismutase active copper chelates but not by native 2Cu,2Zn-superoxide dismutase (cuprein). The ligands alone as well as Cd^{2+}, a heavy metal of similar toxicity to Cu^{2+}, showed no inhibition whatsoever. This indicates that $^{\cdot}O_2^-$ participates in the catalytic cycle and is produced at a site scarcely accessible to such a large molecule as cuprein. A mechanism for the second, aerobic step of the diamine oxidase reaction is suggested.

The participation of superoxide radicals in the catalytic cycles of some oxidases and dioxygenases has been suggested, for example, in the cases of indoleamine 2,3-dioxygenase [1—3] and dopamine β-hydroxylase [4]. The basis of these suggestions was the ability of 2Cu,2Zn-superoxide dismutase (cuprein) to inhibit the reactions catalyzed by these enzymes. In many cases, however, superoxide and/or other excited oxygen species might be produced at a site scarcely accessible to a large molecule like cuprein, which, thus, fails to indicate the participation of the oxygen radicals. The fact that Cu^{2+} and low molecular weight copper chelates exhibit the same superoxide dismutase activity as native cuprein [5—9] made it possible to detect the production of $^{\cdot}O_2^-$ at active sites which could not be reached by superoxide dismutase, i.e. the activation of oxygen by cytochrome *P*-450 during hepatic microsomal dealkylations [8, 10—12].

In the course of the pig kidney diamine oxidase reaction superoxide ions were thought to be produced as could be judged by the initiation of sulfite

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autoxidation and cytochrome *c* reduction [13], but the participation of these oxygen radicals in the conversion of the substrate by the enzyme could not be proved. We report the successful inhibition of the diamine oxidase reaction by superoxide dismutase active copper chelates.

Diamine oxidase was from Sigma Chemicals, München. The superoxide dismutase active copper chelates were synthesized as described previously [5, 6] Superoxide dismutase was isolated from bovine blood [14]. Diamine oxidase activity was assayed spectrophotometrically [15]. 1,4-Diaminobutane hydrochloride served as substrate. The γ-aminobutyraldehyde formed was trapped using *o*-aminobenzaldehyde giving rise to a dye absorbing at 430 nm. The reaction was stopped by adding trichloroacetic acid and, after centrifugation, the concentration of the dye was monitored. All experiments were run in triplicate and the standard error was less than 5%.

The superoxide dismutase active $Cu(tyrosine)_2$ could inhibit the product formation significantly (Fig. 1). While native 2Cu,2Zn-superoxide dismutase did not show any inhibitory action, $Cu(lysine)_2$ and $CuSO_4$, both known to react with superoxide radicals effectively, suppressed the diamine oxidase action (Table I) indicating that the oxygen radicals are formed at a site difficult to reach for the large cuprein molecule.

The ligands alone showed no inhibitory effect. Cd^{2+} ions were used to exclude the possibility that the action of Cu^{2+} is due to heavy metal toxicity. Again, no inhibition whatsoever could be observed even in the presence of 100 μM $CdCl_2$.

The pig kidney diamine oxidase reaction was proposed to occur via a ping-pong mechanism. In the first, anaerobic step, the enzyme-bound pyridoxal phosphate converts amine to aldehyde and is itself transformed to pyridoxamine phosphate. The second, aerobic step is initiated by the formation of a

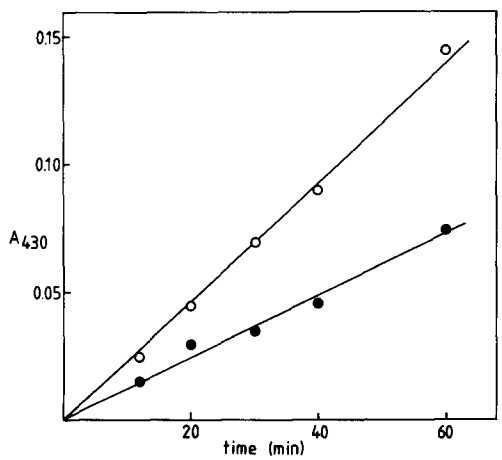

Fig. 1. Effect of $Cu(tyrosine)_2$ on the reaction of diamine oxidase. In a total volume of 5 ml the incubation mixture contained 67 mM phosphate buffer (pH 6.8), 2.5 mM *o*-aminobenzaldehyde, 10.0 mM 1,4-diaminobutane, 180 mU pig kidney diamine oxidase. After incubation at 37° C, the reaction was stopped by adding 1 ml of 10% trichloroacetic acid. After centrifugation the dye formed by the reaction of the generated aldehyde with *o*-aminobenzaldehyde was measured spectrophotometrically at 430 nm against a blank where 1,4-diaminobutane was omitted. ○—○, control; ●—●, + 60 μM $Cu(Tyr)_2$.

TABLE I

INHIBITION OF THE DIAMINE OXIDASE REACTION BY SUPEROXIDE DISMUTASE ACTIVE Cu(II) CHELATES

The reactions were stopped after 40 min. Experimental details are given in the legend to Fig. 1.

Addition	Concentration (μM)	% Inhibition
None	—	0.0
2Cu,2Zn-superoxide-dismutase	0.1	0.0
2Cu,2Zn-superoxide dismutase	1.0	0.0
$Cu(Tyr)_2$	6.0	11.1
$Cu(Tyr)_2$	60.0	48.9
$Cu(Lys)_2$	9.3	6.7
$Cu(Lys)_2$	93.3	34.4
$CuSO_4$	10.0	14.4
$CuSO_4$	100.0	48.9
Tyrosine	100.0	0.0
Lysine	100.0	0.0
$CdCl_2$	100.0	0.0

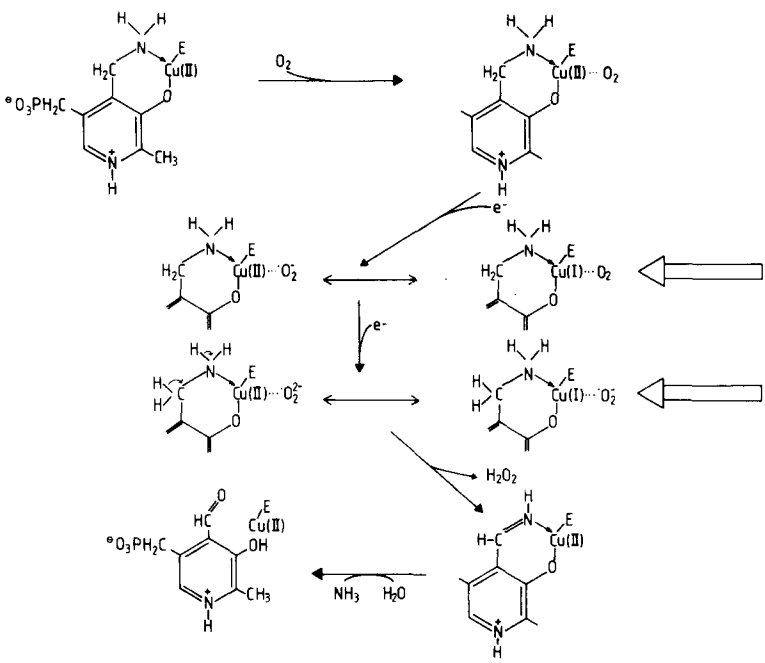

Fig. 2. Suggested mechanism for the second, aerobic step of the diamine oxidase reaction. The possible sites of inhibition by superoxide dismutase active copper chelates are indicated by ⇐.

binary complex with oxygen. After the release of H_2O_2 and NH_3 the native form of the enzyme is regenerated [16]. The enzyme-bound copper is thought to stabilize the co-substrate [17]. Superoxide can only be produced in the second part of the overall reaction, possibly tightly bound to the enzyme. A mechanism is suggested (Fig. 2).

These results show the usefulness of low molecular weight superoxide dismutase active copper chelates as probes to detect the participation of superoxide radicals in cellular biochemistry where the action of native cuprein is

limited due to compartmentation and/or its large molecular dimensions.

This study was alded by DFG grant (We 401/14) awarded to U.W. M.Y. is a recipient of a German Academic Exchange Service (DAAD) fellowship.

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