Inorganica Chimica Acta, 55 (1981) 1–4

Generation of Superoxide Radicals in Alkaline Solutions of Hydrogen Peroxide and the Effect of Superoxide Dismutase in this System

LÁSZLÓ J. CSÁNYI, ZOLTÁN M. GALBÁCS

Institute of Inorganic and Analytical Chemistry, A. József University, 6701 Szeged, Hungary

and LÁSZLÓ HORVÁTH

Institute of Biophysics, Biological Research Center Hungarian Academy of Sciences, 6701 Szeged, Hungary

Received July 31, 1980

It is shown that alkaline hydrogen peroxide cannot serve as a source of superoxide radicals without the presence of a catalyst. Addition of superoxide dismutase to alkaline hydrogen peroxide solution results in an immediate drop in the concentration of superoxide radicals, followed by an increase in the radical yield, because the enzyme is attacked by OH radicals and transformed into an active (but dismutase-inactive) catalyst of hydrogen peroxide decomposition.

Introduction

In a recent paper by Symonyan and Nalbandyan [1] it was stated: "Superoxide radicals in high concentration were generated from alkaline H_2O_2 without using catalyst or irradiation." We willingly agree with these authors that their method is suitable for the production of superoxide radicals without the extra addition of a catalyst, but we dispute that radical production could be realized in the absence of catalyst. Experimental evidence and further comments will be reported below.

Results and Discussion

Although hydrogen peroxide is an unstable molecule from a thermodynamic point of view, its spontaneous decomposition (dismutation) into dioxygen and water is very slow (if it takes place at all) if catalyzing impurities have been thoroughly removed from the aqueous solution [2, 3]. Symonyan and Nalbandyan [1] reported that the iron and copper contents in hydrogen peroxide were found to be lower than the detection limits of these ions using *o*-phenanthroline and bathocuproin disulphonate reagents. We agree that the hydrogen peroxide is not the main source of impurities. Unfortunately, however, it does not seem to have been considered by these authors that all preparations of alkali metal hydroxides contain heavy metal impurities, as can be seen from the labels of potassium hydroxide preparations (Table I).

TABLE I. Data of Labels of Alkali Metal Hydroxides Produced by Different Manufactures.

Impurity	Quantity ($\mu g\ g^{-1}$)				
	BDH Aristar KOH	Chemapol KOH	Merck KOH	Merck NaOH	USSR KOH
Fe	2	5	5	5	5
Cu	0.5		1	0.5	
Mn	0.05		0.5	0.5	
Ni	1		1	5	
Ag	0.05				
heavy metals*		10		5	10

*All heavy metal ions which can be precipitated by hydrogen sulphide.

Figure 1 shows that the rate of decomposition of hydrogen peroxide depends considerably on the source of the potassium hydroxide used to adjust the initial pH of the hydrogen peroxide solution. A considerable drop in the decomposition rate can be observed when the alkali metal hydroxide solutions are subjected to efficient purification [2, 3] (*e.g.* heavy metal impurities are collected on the surface of magnesium hydroxide precipitated in the alkali metal hydroxide solutions [4]).

Our observations indicated that in the presence of different metal ions in trace ($<10^{-5}$ *M*) quantities the rate maxima of hydrogen peroxide decomposition were found at different pHs, *e.g.* pH 9.6, 11.4 and 12.3 if manganese(II), iron(III) and copper(II) catalysts, respectively, were used.

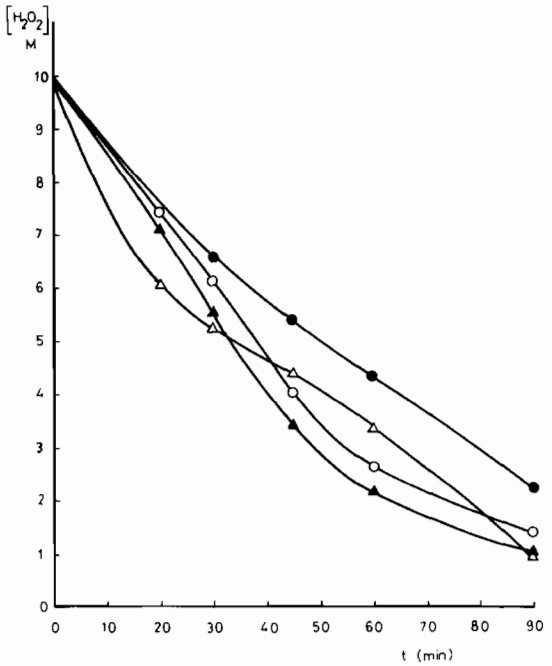

Fig. 1. Decomposition of hydrogen peroxide in alkaline solution at 308 K. Initial pH 8.97 was adjusted by adding alkali metal hydroxide solutions of different source. ●——●, KOH Chemapol G. R.; △——△, NaOH Merck; ○——○, KOH Reanal purified according to [4]; ▲——▲, KOH Reanal.

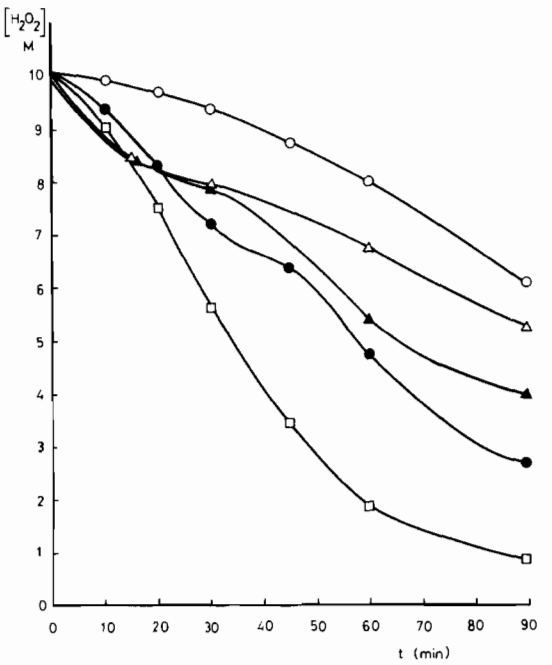

Fig. 2. Influence of chelating agents on the decomposition of hydrogen peroxide in alkaline solution at 308 K. Initial pH 9.03 adjusted by adding KOH (Reanal) solution. ○——○, 10^{-2} *M* EDTA; △——△, 10^{-2} *M* NTA; ▲——▲, 10^{-2} *M* DCTA; ●——●, 10^{-2} *M* EGTA; □——□, without chelate-forming substances.

It was stated in the cited paper that "... the addition of all the chelators, besides EDTA, does not change the observed kinetic curves. Only EDTA (1×10^{-2} *M*) was capable of stopping further increase of pH, temperature and generation of superoxide radicals. This effect of EDTA should not be connected with its chelating ability." The role of chelating agents is quite involved in such a system. The effects of chelators depend strongly on the pH, the concentrations of chelate-forming substances and of metal impurities to be sequestered, and the time, especially when polybases (metal hydroxides) should be removed. It can be seen in Fig. 2 that EDTA is an effective inhibitor of the decomposition of hydrogen peroxide. It exerts its effect almost immediately, although the decomposition is accelerated after a while owing to the destruction of EDTA by the OH radicals formed. NTA (nitrilo-trisacetic acid) is not as effective as EDTA, and exerts its inhibiting effect only after about 15 min. The behavior of DCTA (1.2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid) is quite similar to that of NTA. EGTA (ethyleneglycol-bis(β-aminoethyl ether)) is really not an effective chelator in this system, though its influence on the rate can also be established. It should also be added that the rate-influencing effect of a given chelator can be positive or negative, depending on the concentration in which it is applied [5, 6].

In the Discussion of the cited paper it is stated: "The sharp increase of superoxide radical concentration at pH values above 11.0 agrees with pK = 11.6 for H_2O_2. Thus deprotonation of peroxide is essential for the generation of superoxide radicals". If the pH-dependence of the decomposition of hydrogen peroxide catalyzed by copper(II) ions is compared with the pH-dependence of the ESR signal intensity (Fig. 3), one has to conclude that superoxide radical production is connected with copper(II)-catalyzed hydrogen peroxide decomposition.

It was earlier demonstrated that dismutation of superoxide radicals can be effectively catalyzed by copper(II) ions [7]. This is also supported by the findings of Symonyan and Nalbandyan. However, it does not follow from this fact that the decomposition of hydrogen peroxide resulting in superoxide radicals cannot be catalyzed by copper(II) ions more effectively.

The observation [1] that an increase of one pH unit results in a thousandfold increase in the superoxide radical yield can be regarded as convincing evidence of the presence of catalysts in the system. To adjust the initial pH of 30% hydrogen peroxide to 10 instead of 9 it is necessary to add about ten times more alkali metal hydroxide, which is equivalent to a tenfold increase in the concentration of catalyzing impurities. Therefore, a tenfold increase in

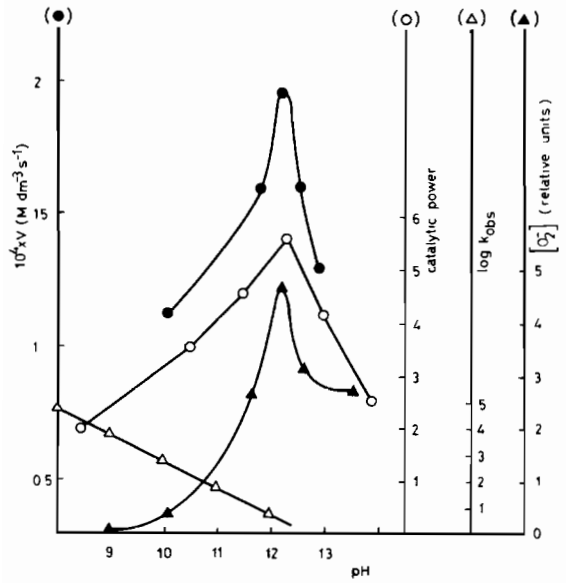

Fig 3 Comparison of the pH-dependences ●——●, rate of decomposition of hydrogen peroxide (1 0 *M*) catalysed by 10^{-6} *M* copper(II) ions at 308 K, ○——○, catalytic power of superoxide dismutase according to [1], △——△, rate of uncatalysed dismutation of superoxide radical [8], ▲——▲, concentration of superoxide radicals formed in 5 *M* H_2O_2 solutions after about 4 min having the pH adjusted by KOH (Reanal) to the appropriate values The ESR spectrum was recorded near to liquid N_2 temperature

the radical concentration is to be expected However, it must also be considered that, with the increase of the initial pH, not only does the concentration of impurities increase, but the activity of the catalyst is also influenced substantially Further, the rate of uncatalyzed dismutation of superoxide radicals also decreases by a factor of 10 per pH unit increase [8]

The data in Table I of [1] show that the catalytic activity of superoxide dismutase changes with the pH according to a curve having a maximum at pH 12 3 (Fig 3, curve ○——○) However, considering that both copper(II)-catalyzed hydrogen peroxide decomposition and superoxide radical production display a maximum at that very pH, we are of the opinion that here the pH-dependence of radical production much rather than the pH-dependence of enzyme activity is involved This view is supported by the experimental procedure [1], as the concentration of superoxide radicals was measured before and immediately after the addition of the enzyme, consequently, one should find a maximum in the difference of concentration of superoxide radicals at that pH where the radical production exhibits a maximum, since the removal of radicals by the enzyme takes place at a constant rate (if the finding that the enzymatic dismutation is independent of pH in the range 5 0–9 5 [9, 10] is extrapolated to higher pH values), or at a diminishing rate if both the denaturation of the enzyme in strongly alkaline solution and the inactivator role of concentrated hydrogen peroxide [11] are taken into consideration

To finish we should like to comment on the role of superoxide dismutase in this system It was stated in [1] that "The addition of superoxide dismutase to alkaline H_2O_2 led initially to a drop in the EPR spectrum intensity, followed by an increase in the concentration of superoxide radicals" This rather peculiar 'double' role of the enzyme can be explained more simply if it is considered that OH radicals are formed during the catalysed decomposition of hydrogen peroxide In the presence of superoxide dismutase one has to assume that not only hydrogen peroxide but also the enzyme is attacked by OH radicals The destruction of the enzyme results in a drop in the dismutase activity, but at the same time fragments containing copper(II) are released, which stimulate O_2^- production by enhancing the rate of decomposition of hydrogen peroxide

Experimental

Materials

'Perhydrol' (Merck) hydrogen peroxide and other chemicals of c p grade were used without further purifications KOH (Reanal) was purified according to [4] Triply distilled water was used to prepare the solutions

Methods

The pH of the reaction mixture was adjusted by adding purified KOH solution To avoid the contamination of reaction mixtures 2–3 ml samples were periodically poured into dry beakers and from them 0 5 ml was pipetted and added to acidified 50 ml *ca* 0 2 N arsenite solution Then 1 drop of 0 01 *M* OsO_4 catalyst and 1 drop of 0 025 *M* ferroin indicator solution were added and the solution was backtitrated by 0 1 *M* standardized cerium(IV) sulphate solution The decomposition of hydrogen peroxide was also followed by measurement of O_2 evolved Figures furnished by the two methods agree sufficiently well The slope of the concentration *vs* time curve was regarded as the initial rate of decomposition

The concentration of O_2^- was determined by ESR spectrometry in frozen samples and the spectra were recorded by a JEOL-JES PE spectrometer near to liquid nitrogen temperature

References

1 M A. Symonyan and R M. Nalbandyan, *Biochim Biophys Acta, 583,* 279 (1979)

2 F R Duke and T W Haas, *J Phys Chem*, *65*, 304 (1961)
3 E Koubek, M L Haggett, C J Battaglia, Ibne-Rasa, M Khairat, H Y Pyun and J O Edwards, *J Am Chem Soc*, *85*, 2263 (1963)
4 J D'Ans and J Mattner, *Angew Chem*, *64*, 448 (1952)
5 H Sigel and T Kaden, *Helv Chim Acta*, *51*, 947 (1968)
6 M T Beck, S Gorog and Z Kiss, *Acta Chim Hung*, *42*, 321 (1964)
7 J Rabani and D Klug-Roth, *J Phys Chem*, *77*, 1169 (1973)
8 Bielski, H J Benon and A O Allen, *J Phys Chem*, *81*, 1048 (1977)
9 D Klug, J Rabani and I Fridovich, *J Biol Chem*, *247*, 4839 (1972)
10 B G Malmstrom, L E Andréasson and B Reinhammar, in 'The Enzymes', P D Boyer ed, Vol 12, third ed, pp 507–580, Academic Press, New York, 1975
11 I Fridovich, in 'Free Radicals in Biology', W A Pryor ed, Vol 1, pp 239–271, Academic Press, New York, 1976