MODE OF SINGLET OXYGEN INDUCED CHEMILUMINESCENCE IN THE PRESENCE OF ERYTHROCUPREIN

Ulrich WESER and Wulf PASCHEN
Physiologisch-Chemisches Institut der Universität Tübingen, Germany

Received 21 August 1972

1. Introduction

An overwhelming number of studies on the long known copper protein called erythrocuprein [1] have been reported in recent years. A summary of the basic structural and biochemical principles will soon be published [2]. This copper and zinc containing metalloprotein displays an important biochemical function, namely it accelerates the disproportionation of univalent superoxide anion radicals into H_2O_2 and O_2 [3, 4]. Due to this biochemical reactivity erythrocuprein was renamed superoxide dismutase. On the other hand, the scavenging of singlet oxygen by erythrocuprein was considered of utmost importance [5]. During the enzymic catalysed oxidation of xanthine singlet oxygen was detected by measuring the subsequent chemiluminescence in the presence of luminol. Usually, the chemiluminescence was recorded using a scintillation counter. In those chemiluminescent assays described elsewhere [5, 6] the coincidence circuit was deliberately turned off to increase the yield of the light emission. However, we were especially interested in the mode of light emission under minimized interferences from other sources. Furthermore, a stronger chemiluminescence was expected at pH values >8.0 due to the increased stability and yield of $O_2^-\cdot$ [7].

The inhibition of the chemiluminescence caused by singlet oxygen was studied in the presence of erythrocuprein. At pH 7.9 ± 0.1 a normal decay curve was detectable. At higher pH the initial light emission was followed by a distinct maximum which was shifted towards a longer time. In the presence of erythrocuprein this maximum appeared much earlier. The relative inhibition of the chemiluminescence was smaller at pH 9.0. A quantitative evaluation of erythrocuprein concentrations using this scintillation method was possible and gave linear calibration curves.

2. Experimental

Bovine erythrocuprein was prepared from fresh erythrocytes using organic solvents, gel- and DEAE 23 chromatography [2, 3]. Singlet oxygen responsible for the chemiluminescence was produced using the xanthine-xanthine oxidase system in the presence of luminol. Light emission was measured in a Packard scintillation

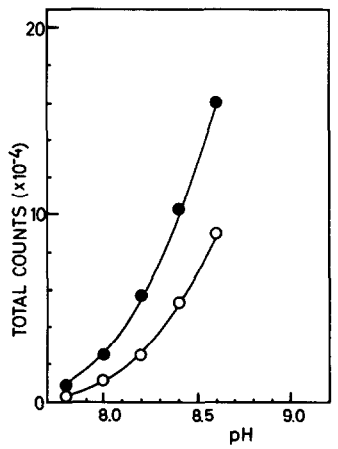

Fig. 1. Total light emission without (•–•–•) and with 2.2 nM erythrocuprein (o–o–o). Each scintillation vial contained in a volume of 2.11 ml: Xanthine 4.5 mM; luminol, 1 mM; catalase 1.4 × 10^3 I.U.; HEPES buffer 50 mM. The reaction was started with 50 µl xanthine oxidase (0.04 units as defined in [8]. The counting time was 10 min.

North-Holland Publishing Company – Amsterdam

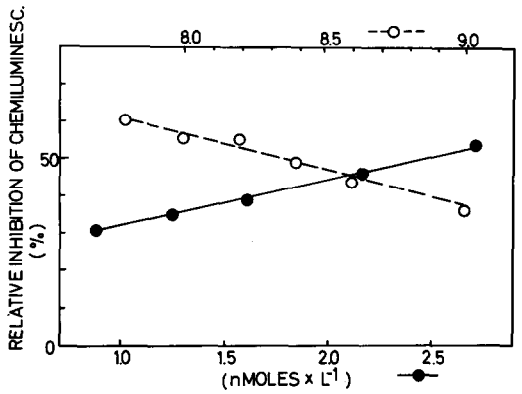

Fig. 2. Relative inhibition of singlet oxygen formation in the presence of different erythrocuprein concentrations (•—•—•). Alternatively, the pH dependence at a constant erythrocuprein concentration (3.2 nM) is depicted (o—o—o). The assay conditions were exactly the same as in fig. 1.

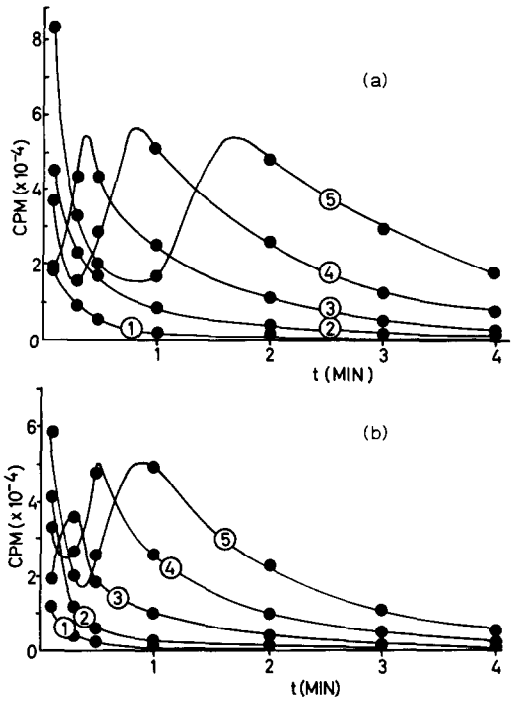

Fig. 3. pH and time dependent velocity of light emission in the absence (a) and presence (b) of erythrocuprein. 2.11 ml of the scintillation assay mixture were composed of: xanthine, 4.5 mM; luminol, 1 mM; catalase, 1.4 × 10^3 I.U.; HEPES buffer, 50 mM; start with 50 µl xanthine oxidase (0.04 units per definition as in [8]). ①pH 7.8; ② pH 8.0; ③ pH 8.2; ④ pH 8.4 and ⑤ pH 8.6. Counting was performed in dim daylight and the coincidence circuit was turned on to minimize interferences from outside.

counter (model 2002). The setting was exactly as for ^{3}H-counting. The coincidence circuit was turned on. The reaction was started with xanthine oxidase. The first reading was taken after 10 sec. The counting rate was over 50 000 cpm. The control value omitting xanthine oxidase was 5 ± 3 cpm.

3. Results

Upon adjusting the pH to 8.6 the light emission was 16 times higher compared to the corresponding measurements at pH 7.8 (fig. 1). Added erythrocuprein reduced this scintillation markedly. The relative inhibition was more expressed at pH 8.0 ± 0.2 (figs. 1 and 2). Under the present conditions the diminished light emission was linear to different erythrocuprein concentrations.

A most interesting phenomenon was observed when the velocity of the light emission (expressed as cpm) was plotted versus the time. At pH 7.9 ± 0.1 a normal decay curve can be seen. Starting from pH 8.2 both a different time course and a higher yield of the light emission is apparent. The initial decay of the light emission was followed by a distinct maximum which was shifted towards a longer time at increasing pH values. In the presence of erythrocuprein the initial light emission was strongly diminished and the maximum was shifted towards a shorter time. However, it has to be emphasized that the overall characteristics of the time dependent scintillation is virtually the same, except that the yield of the light emission is markedly reduced.

4. Discussion

Omission of catalase did not affect the mode of the time dependent light emission except the maximum appeared at a later time [9]. The question arises after the origin of this maximum. It could be possible that substrate inhibition of the xanthine oxidase by $O_2^-\cdot$ or O_2^{2-} or a delay in the luminol reaction is responsible

for this phenomenon. Furthermore this mode of light emission is substantially different from similar studies where the coincidence circuit was turned off [5, 6]. In these investigations a plateau appeared after some 10 min which was followed by a gradual decay. It should be emphasized that under the present assay conditions where unspecified interferences were minimized the light emission is much more expressed at elevated pH values. This is in contrast to the finding of Agrò et al. [5] who used the experimental setting reported in [6, 10] with the consequence of a marked reduction of the chemiluminescence at pH 9.0.

Acknowledgements

This study was supported by a DFG grant No. 401/4 donated to U.W.

References

[1] T. Mann and D. Keilin, Proc. Roy. Soc. London, Ser. B. 126 (1939) 303.
[2] U. Weser, Structure & Bonding, Berlin, in press.
[3] J.M. McCord and I. Fridovich, J. Biol. Chem. 244 (1969) 6049.
[4] L. Fridovich, J. Biol. Chem. 245 (1970) 4053.
[5] A. Finazzi-Agrò, C. Giovagnoli, P. De Sole, L. Calabrese, G. Rotilio and B. Mondovi, FEBS Letters 21 (1972) 183.
[6] R.M. Arneson, Arch. Biochem. Biophys. 136 (1972) 352.
[7] D. Ballou, G. Palmer and V. Massey, Biochem. Biophys. Res. Commun. 36 (1969) 898.
[8] J. Cooper, P.A. Srere, M. Tabachnick and E. Racker, Arch. Biochem. Biophys. 74 (1958) 306.
[9] K.E. Joester, Biochemical Masters Thesis, Univ. Tübingen, 1972.
[10] J.R. Vorhaben and R.H. Steele, Biochemistry 6 (1967) 1404.