# DEPENDENCE OF THE DICARBONYL-SENSITIZED PHOTOINACTIVATION OF LACTOPEROXIDASE ON IRRADIATION WAVELENGTH

K. K. MÄKINEN and P.-L. MÄKINEN*

*Department of Biochemistry, Institute of Dentistry, University of Turku, 20520 Turku 52* and **Department of Forensic Medicine, University of Turku, 20500 Turku 50, Finland*

Received 16 December 1981

## 1. Introduction

It has been known for more than 70 years that carbonyl compounds act as sensitizers in photochemical reactions [1] and for more than 40 years that 2,3-butanedione (biacetyl) readily undergoes photochemical reactions in the visible region [2]. In spite of these facts, BD has been exploited as a specific probe for active arginyl residues under strong photooxidative conditions. It was demonstrated that the BD-sensitized photochemical inactivation of an enzyme leads to extensive structural changes in the enzyme molecule regardless of the essentiality or unessentiality of arginyl residues for enzyme activity [3–8]. Continuation studies in this laboratory revealed that other diketones and ketone aldehydes, previously used as probes for active arginine, also caused similar extensive structural changes in the enzyme molecule.

This study shows for the first time the wavelengths at which maximum photochemical inactivation of an enzyme, sensitized by different carbonyl compounds, takes place. It was also shown that in addition to BD, also CHD, PD, PPD, PG and MG photoinactivate bovine milk lactoperoxidase which was used as a model enzyme. BD, PPD and PD inactivated LPO both in the UV and visible range, whereas CDH, PG and MG inactivated in the UV range only. The most effective photosensitizing agent was CHD. These irreversible processes proceed regardless of the importance or unimportance of arginyl residues for enzyme activity.

*Abbreviations:* BD, 2,3-butanedione; CHD, 1,2-cyclohexanedione; PD, 2,3-pentanedione; PPD, 1-phenyl-1,2-propanedione; PG, phenylglyoxal; MG, methylglyoxal; LPO, bovine milk lactoperoxidase (EC 1.11.1.7)

## 2. Materials and methods

### 2.1. *Chemicals*

2,3-Butanedione was purchased from Koch-Light (Colnbrook, Buckinghamshire). Methylglyoxal and 1-phenyl-1,2-propanedione were obtained from Sigma (St Louis MO). Phenylglyoxal and 1,2-cyclohexanedione were from Aldrich-Europe (Beerse) and 2,3-pentanedione was a product of Fluka (Buchs). All dicarbonyl compounds were stored between 0–5 °C in the dark and they were used within a few days after purchase. Bovine milk lactoperoxidase was obtained from P-L Biochemicals (Milwaukee WI). The sources of other reagents were mentioned in [6,8].

### 2.2. *Determination of LPO activity*

The activity of LPO was determined using guaiacol or 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid) as the substrate [8–10].

### 2.3. *Performance of photooxidation of LPO*

The photooxidation of LPO in the presence of dicarbonyl compounds was in principle carried out as in [5,6,8]. However, the modification mixtures containing 10 mM phosphate buffer (pH 7.0) and 0.45 μM LPO, were irradiated in quartz cells in a thermostated Hitachi MPF-2A fluorescence spectrophotometer (with the filter between the cell and the excitation monochromator removed) at different wavelengths (indicated below). The bandwidth was 40 nm. The appropriate controls were run under identical conditions. The fluorescence spectra of LPO treated in the absence and in the presence of the sensitizer were also recorded with the above instrument. Ultraviolet and visible absorption spectra were recorded with a Perkin Elmer



*Published by Elsevier Biomedical Press*
00145793/82/0000–0000/$02.75 

double beam spectrophotometer 124. The rate constants of inactivation of LPO were calculated from the equation $k_{app} = \ln(A_1/A_2)/(T_2-T_1)$ where $A_1$ is the original enzyme activity determined at time $T_1$ and $A_2$ is enzyme activity after 10–30 min ($T_2$) illumination. The final values of $k_{app}$, given in fig.2, were calculated as arithmetic means of 4 expt. In quenching experiments the quencher was added into the modification mixture 5 min before irradiation was started. Treatment of PLO in an anoxic medium (under $N_2$) was conducted as in [6,8].

## 3. Results

LPO was irradiated at 280 nm in the presence of 6 different carbonyl compounds (1 mM; fig.1A). These

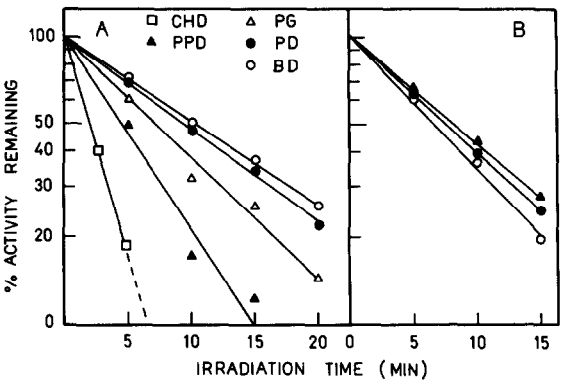


Fig.1. Dicarbonyl-sensitized photochemical oxidation of LPO: $4.5 \times 10^{-7}$ M LPO was treated with 1 mM dicarbonyl compounds in 0.4 ml reaction mixtures; 5 μl aliquots were withdrawn at the times indicated for activity assays; (A) irradiation at 280 nm; (B) irradiation at 410 nm.

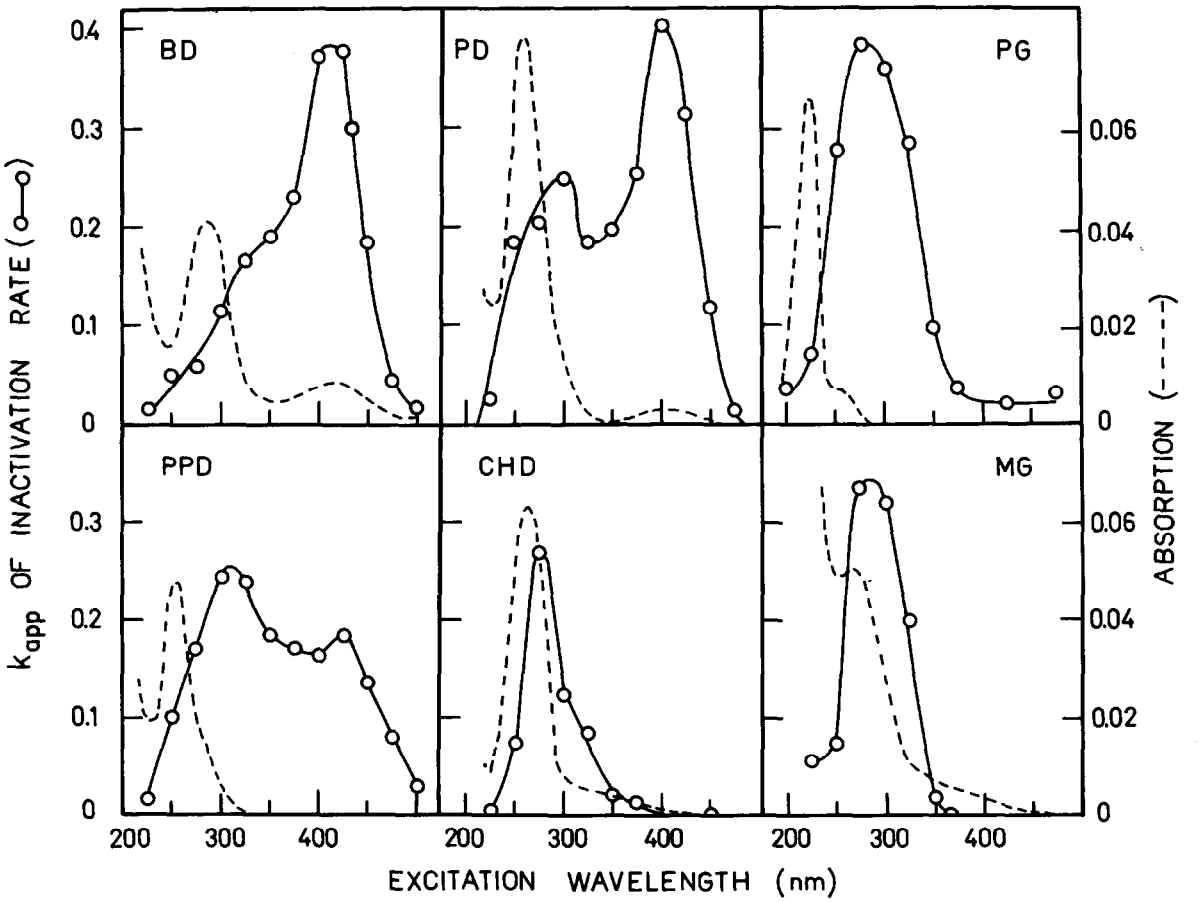


Fig.2. Dicarbonyl-sensitized photochemical oxidation of LPO as a function of irradiation (excitation) wavelength (o): $4.5 \times 10^{-7}$ M LPO was treated for 20 min with the dicarbonyl compounds shown in 0.4 ml reaction mixtures; 5 μl aliquots were withdrawn at the times indicated for activity assays. The sensitizer concentrations (0.25–2.7 mM) were chosen to yield a suitable rate of inactivation of LPO in each case. The absorption curves (– – –) of the sensitizers are also shown. The absorption was measured both in methanolic and aqueous solutions. Both measurements produced similar absorption curves.

treatments caused a rapid and irreversible inactivation of LPO. For example, 1 mM CHD brought about a total loss of LPO activity in <10 min, when the molar ratio of the sensitizer to the enzyme (CHD/LPO) was 2500. The half-time of inactivation varied as a function of the concentration of all sensitizers, as found with BD [5]. Out of the carbonyl compounds tested at 280 nm, BD, PD, and PPD inactivated LPO in the visible region as well (fig.1B).

Tha ability of the dicarbonyls to inactivate LPO depended on irradiation wavelength (fig.2). PD and PPD showed two distinct maxima, whereas BD, CHD, PG and MG had one inactivation maximum only. The shoulder on the left limb of the BD curve indicated considerable inactivation of LPO in the UV range as well. Maximum inactivation of LPO occurred at or close to the absorption maxima of the carbonyl compounds tested (fig.2).

When the LPO–dicarbonyl mixtures were irradiated in the presence of 1 mM $NaN_3$, various imidazole derivatives (2-thiol-L-histidine, 3-methyl-L-histidine and related compounds [6–8], or under $N_2$, no inactivation of LPO took place. After the protector was consumed during irradiation, inactivation progressed rapidly. The protectors (quenchers) were effective both in UV and visible region. These experiments suggest that the inactivation of LPO by the dicarbonyl compounds tested was a photochemical process.

The dicarbonyl-sensitized photochemical inactivation of LPO led to extensive structural changes in the enzyme molecule. These changes qualitatively resembled those of *Aeromonas* aminopeptidase, observed in the presence of BD [5]. The most dramatic change was the virtually complete loss of tryptophan fluorescence of LPO. The loss of fluorescence proceeded faster than the loss of enzyme activity, suggesting that tryptophyl residues would not be of primary importance for LPO activity. This idea was supported by studies which showed that 2-hydroxy-5-nitrobenzyl bromide did not inhibit LPO [8]. The loss of tryptophan fluorescence could be prevented by keeping the reaction mixture in anoxic media ($N_2$) or by adding singlet oxygen quenchers or free radical scavengers into the irradiation mixture. The UV and visible spectra of LPO were also affected. These changes were detailed in [8]. In addition to acting as photosensitizers of LPO, the dicarbonyls may also have reacted irreversibly with certain arginyl residues of LPO, thereby increasing absorption of LPO in the UV region. Absorption studies in the visible region suggested that the photochemical oxidation also affected the binding of heme to the apoenzyme.

## 4. Discussion

BD cannot be regarded as a suitable probe for active arginyl residues of enzymes if the modification experiments are carried out in photooxidative conditions [3–8]. This study demonstrates that other widely exploited arginine reagents, PG and CHD, and others that in theory could be used for this purpose (PD, PPD and MG), are related, general photooxidizing agents. The ability of CHD in particular to cause a rapid and irreversible inactivation of LPO was remarkable. The inactivation mechanism may include formation of singlet oxygen ($^1\Delta_g$), free radicals and other active species from the sensitizer [7]. The formation of free radicals from BD has been described [5]. The formation of free radicals, other active species or $^1\Delta_g$ from the other dicarbonyls used is also possible. Such active species are able to react with protein tryptophyl, tyrosyl, histidyl, sulfhydryl and other groups [7,8], leading to extensive structural changes in the enzyme [3–8]. This fact suggests that conclusions from enzyme modification data obtained with dicarbonyl compounds can be drawn only if the illumination conditions are carefully controlled. The wavelength range in which BD, PD and PPD caused significant photoinactivation was remarkably wide. This indicates that in enzyme modification experiments with these dicarbonyl compounds, virtually all illumination should be avoided.

## References

[1] Berthelot, D. and Gaudechon, H. (1910) Compt. Rend. 151, 478–481.
[2] Roof, J. G. and Blacet, F. E. (1941) J. Am. Chem. Soc. 63, 1126–1128.
[3] Fliss, H. and Viswanatha, T. (1979) Can. J. Biochem. 57, 1267–1272.
[4] Gripon, J. C. and Hofman, T. (1981) Biochem. J. 193, 55–65.
[5] Mäkinen, K. K. (1981) FEBS Lett. 131, 165–168.
[6] Mäkinen, K. K. Mäkinen, P.-L., Wilkes, S. H., Bayliss, M. E. and Prescott, J. M. (1982) J. Biol. Chem. in press.
[7] Mäkinen, K. K. and Mäkinen, P.-L. (1982) submitted.
[8] Mäkinen, K. K. and Mäkinen, P.-L. (1982) Eur. J. Biochem. in press.
[9] Chance, B. and Maehly, A. C. (1964) Methods Enzymol. 2, 764–775.
[10] Shindler, J. S., Childs, R. E. and Bardsley, W. G. (1976) Eur. J. Biochem. 65, 325–331.