Biochemical and Biophysical Research Communications **282,** 380–386 (2001)
doi:10.1006/bbrc.2001.4582, available online at http://www.idealibrary.com on IDEAL®

Mechanism of Reaction of Melatonin with Human Myeloperoxidase

Mario Allegra,* Paul Georg Furtmüller,† Günther Regelsberger,† Maria L. Turco-Liveri,‡ Luisa Tesoriere,* Mauro Perretti,§ Maria A. Livrea,* and Christian Obinger†,[1]

*Department of Pharmaceutical Toxicological and Biological Chemistry, University of Palermo, Via Carlo Forlanini, 90123 Palermo, Italy; †Institute of Chemistry, University of Agricultural Sciences, Muthgasse 18, A-1190 Vienna, Austria; ‡Department of Physical Chemistry, University of Palermo, Viale delle Scienze, 90100 Palermo, Italy; and §Department of Biochemical Pharmacology, W. Harvey Research Institute, Queen Mary and Westfield College University of London, London, United Kingdom

Received February 12, 2001

Recently, it was suggested that melatonin (*N*-acetyl-5-methoxytryptamine) is oxidized by activated neutrophils in a reaction most probably involving myeloperoxidase *(Biochem. Biophys. Res. Commun.* (2000) 279, 657–662). Myeloperoxidase (MPO) is the most abundant protein of neutrophils and is involved in killing invading pathogens. To clarify if melatonin is a substrate of MPO, we investigated the oxidation of melatonin by its redox intermediates compounds I and II using transient-state spectral and kinetic measurements at 25°C. Spectral and kinetic analysis revealed that both compound I and compound II oxidize melatonin via one-electron processes. The second-order rate constant measured for compound I reduction at pH 7 and pH 5 are (6.1 ± 0.2) × 10^6 M^{-1} s^{-1} and (1.0 ± 0.08) × 10^7 M^{-1} s^{-1}, respectively. The rates for the one-electron reduction of compound II back to the ferric enzyme are (9.6 ± 0.3) × 10^2 M^{-1} s^{-1} (pH 7) and (2.2 ± 0.1) × 10^3 M^{-1} s^{-1} (pH 5). Thus, melatonin is a much better electron donor for compound I than for compound II. Steady-state experiments showed that the rate of oxidation of melatonin is dependent on the H_2O_2 concentration, is not affected by superoxide dismutase, and is quickly terminated by sodium cyanide. Melatonin can markedly inhibit the chlorinating activity of MPO at both pH 7 and pH 5. The implication of these findings in the activated neutrophil is discussed.

Key Words: **melatonin; myeloperoxidase; compound I; compound II; transient-state kinetics, chlorination activity.**

Melatonin (*N*-acetyl-5-methoxytryptamine), one of the biologically relevant indole compounds, is a hormone secreted by the pineal gland of vertebrates, which controls several physiological functions associated with circadian and seasonal rhythms (1). Since it is an evolutionary highly conserved molecule and is ubiquitously present in organisms whose metabolism is based on oxygen, it has been speculated that a primary function of melatonin is the detoxification of reactive oxygen intermediates (2, 3). Besides blood, melatonin was reported to be found and synthesized in a variety of tissues including bone marrow (4, 5) in relatively high concentrations. There is also evidence that melatonin participates in the immune response effecting leukocyte activation and cytokine production (6, 7). Recently, it was shown that melatonin is oxidized by activated neutrophils most probably involving myeloperoxidase (8). That myeloperoxidase (MPO) participates in the formation of N^1-acetyl-N^2-formyl-5-methoxykynuramine (an oxidation product of melatonin) was deduced from the strong inhibitory effect of azide on the oxidation reaction (8).

Myeloperoxidase is the most abundant protein of neutrophils, which are our primary defenders against invading pathogens and also are among the major effector cells in numerous inflammatory pathologies (9). During its catalytic cycle, the ferric or native myeloperoxidase reacts with hydrogen peroxide forming compound I (MPO-I) (reaction [1]). This redox intermediate is known to oxidize halides via a single two-electron reaction to produce the respective cytotoxic hypohalous acids and regenerate the native enzyme (reaction [2]). Alternatively, stepwise reduction of compound I by two donor-derived electrons produces compound II (MPO-II) and subsequently the resting ferric state (reactions [3] and [4]).

Abbreviations used: MPO, myeloperoxidase; MPO-I, compound I; MPO-II, compound II; AFMK, N^1-acetyl-N^2-formyl-5-methoxykynuramine; DMF, dimethylformamide.

[1] To whom correspondence should be addressed. Fax: +43-1-36006-6059. E-mail: cobinger@edv2.boku.ac.at.

0006-291X/01 $35.00

$$MPO + H_2O_2 \leftrightarrow MPO\text{-}I + H_2O \quad [1]$$

$$MPO\text{-}I + X^- \leftrightarrow MPO + HOX \quad [2]$$

$$MPO\text{-}I + AH_2 \rightarrow MPO\text{-}II + AH^\cdot \quad [3]$$

$$MPO\text{-}II + AH_2 \rightarrow MPO + AH^\cdot \quad [4]$$

Since recent evidence has shown that melatonin can reduce hypervalent iron species similar to those involved in the catalytic cycle of MPO (10), in the present paper we have investigated the reaction of melatonin with both redox intermediates of MPO by using multimixing stopped-flow spectroscopy, in order to clarify if myeloperoxidase-mediated melatonin oxidation could be of physiological relevance.

We show that melatonin is an excellent substrate for MPO-I, but reacts slowly with MPO-II. Melatonin oxidation occurs during the peroxidatic cycle of MPO in two one-electron oxidation steps (reactions [3] and [4]). We report actual bimolecular rate constants for reactions of compounds I and II with melatonin at pH 5 and 7, as well as the influence of melatonin on the *in vitro* chlorination activity of MPO at pH 5 and 7. The biological implication of this reaction is discussed.

MATERIALS AND METHODS

Materials

Highly purified myeloperoxidase of a purity index (A430/A280) of at least 0.85 was purchased from Planta Naturstoffe Vetriebs GmbH. Determination of its concentration and that of hydrogen peroxide was performed as reported previously (13). Hydrogen peroxide was prepared shortly before use. Melatonin was dissolved in dimethylformamide (DMF) and finally diluted with phosphate buffer (pH 7) or phosphate/citrate buffer (pH 5). The final DMF concentration was 2% ($^v/_v$) in all assays. All chemicals were purchased from Sigma Chemical Co. at the highest grade available.

Methods

Stopped-flow spectroscopy. The sequential stopped-flow apparatus (Model SX-18MV) and the associated computer system were from Applied Photophysics (UK). For a total of 100 μl/shot into a flow cell with 1 cm light path, the fastest time for mixing two solutions and recording the first data point was ca. 1.5 ms. Because of the inherent instability of MPO compound I (11), sequential stopped-flow (multimixing) analysis was used for determination of rates of the reaction of compound I with melatonin. The reactions were followed at the Soret maximum of compound II (456 nm). In a typical experiment MPO (4 μM heme) in 5 mM buffer (pH 7) was premixed with 40 μM H_2O_2 in distilled water. After a delay time of 20 ms, compound I was allowed to react with varying concentrations of melatonin in 200 mM phosphate buffer (pH 7) or phosphate/citrate buffer (pH 5). The final DMF concentration was 2% (v/v). The pH after mixing was measured at the outlet.

Reduction of compound II by melatonin was performed as described recently (12). In a typical experiment MPO (4 μM heme) in 5 mM buffer (pH 7) was premixed with 40 μM H_2O_2 and 1.8 μM homovanillic acid in distilled water. After a delay time of 40 s, compound II was allowed to react with varying concentrations of melatonin in 200 mM phosphate buffer or 200 mM phosphate/citrate buffer (pH 5). The reactions were followed at 456 nm (disappearance of compound II) and 430 nm (formation of ferric MPO). At least three determinations (2000 data points) of k_{obs} were performed for each substrate concentration and the mean value was used in a plot of k_{obs} versus substrate concentration. The final melatonin concentrations were at least 10 times in excess of the enzyme to assure first-order kinetics. All reactions were performed at 25°C.

Reduction of both compound I and compound II was also studied with the diode-array detector. Typically, the final heme concentration used in these experiments was 2 μM.

Melatonin assay. Oxidation of melatonin (100 μM) was followed at 340 nm and 25°C using the Zeiss Specord S-10 diode-array spectrophotometer. Assays contained 10-200 nM MPO and 10-100 μM H_2O_2 in 100 mM phosphate/citrate (pH 5) or phosphate buffer (pH 7). The kinetics of oxidation was followed at 340 nm since at this wavelength the absorbance was significantly increased as a function of the incubation time. An increase at 340 nm is consistent with the formation of N^1-acetyl-N^2-formyl-5-methoxykynuramine (8, 13). Reactions were started by addition of hydrogen peroxide and were performed in the absence and presence of CuZn-superoxide dismutase (30 μg/ml).

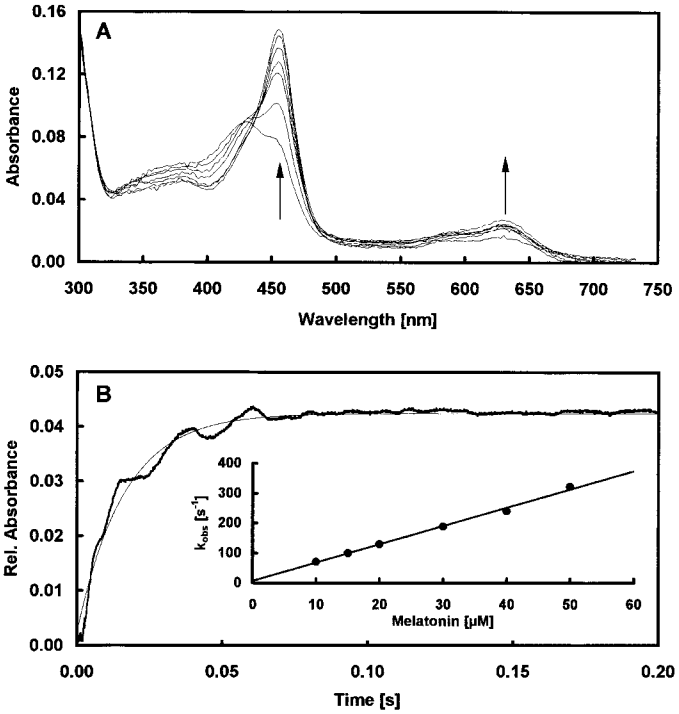
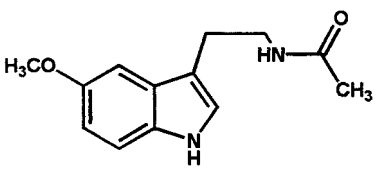
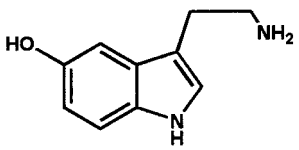
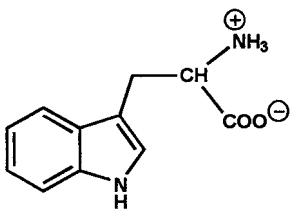
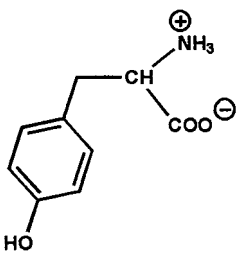


FIG. 1. Reaction of compound I with melatonin. (A) Spectral changes observed when 25 μM melatonin reacted with MPO compound I (2 μM heme). The first scan was taken 1.3 ms after mixing, subsequent scans at 3.8, 6.4, 8.9, 16.6, 40, and 1000 ms. Compound I was formed as described under Materials and Methods. The reaction was carried out in 100 mM phosphate buffer, pH 7. (B) Typical time trace of the reaction followed at 456 nm (10 μM melatonin). Final enzyme concentrations were 1 μM heme in 100 mM phosphate buffer, pH 7. Additionally, the single-exponential fit used for calculation of the pseudo-first-order rate constant is shown. The inset shows pseudo-first-order rate constants for compound II formation plotted against melatonin concentration.

TABLE 1

Summary of Bimolecular Rate Constants for Reactions of Myeloperoxidase Compound I (k_3) and Compound II (k_4) with Indole Derivatives and Tyrosine at pH 7 and 25°C

	k_3 (M^{-1} s^{-1})	k_4 (M^{-1} s^{-1})	Structure	Reference
Melatonin	6.1×10^6	9.6×10^2		This paper
Serotonin	1.7×10^7	2.2×10^6		(17)
Tryptophan	2.1×10^6	7		(18)
Tyrosine	7.7×10^5	1.6×10^4		(16)

Myeloperoxidase-catalyzed chlorination of monochlorodimedon. Monochlorodimedon (MCD) is a substrate often used to study MPO-catalyzed chlorination (14). Chlorination of MCD to dichlorodimedon results in a decrease in absorbance at 290 nm. MCD (100 μM) was dissolved in 100 mM phosphate/citrate buffer (pH 5) or phosphate buffer (pH 7) containing 100 μM H_2O_2 and 100 mM chloride in the presence or absence of melatonin. Reactions were started by addition of MPO (20 nM at pH 5.0, and 200 nM at pH 7.0) and performed in a Beckman DU 640 spectrophotometer equipped with a temperature controller at 25°C.

RESULTS AND DISCUSSION

The mechanism of action of melatonin is poorly known. Systematic studies have provided evidence that melatonin can act as an effective radical scavenger, thus protecting organisms from oxygen radical damage (3). Recent evidence has been provided that melatonin is a good electron donor in the reduction of hemoglobin oxoferryl species (10). This led us to hypothesize that it may also function as a substrate for peroxidases. This work was also motivated by the recent finding that melatonin is oxidized in activated neutrophils, and that myeloperoxidase could be involved in this reaction (8).

Reduction of compound I by melatonin. Using the sequential stopped-flow technique we monitored the spectral changes upon reaction of compound I with melatonin. MPO and H_2O_2 were premixed, resulting in compound I formation as described recently (11, 12). The newly formed compound I was then mixed with melatonin. Figure 1A unequivocally demonstrates the direct conversion of compound I into compound II, which exhibits maxima at 456 nm (Soret peak) and 625 nm. These data suggest a one-electron reduction of compound I to compound II. The absorbance change monitored at 456 nm can be fitted to a single-exponential function (Fig. 1B) and the observed pseudo-first-order rate constants are linearly dependent on the concentration of melatonin (inset to Fig. 1B). The second-order rate constant calculated from such a plot is $(6.1 \pm 0.2) \times 10^6$ M^{-1} s^{-1} at pH 7.0 and

25°C. There is a finite intercept of (6.5 ± 3.6) s^{-1}. This is a similar value as determined for the reactions of compound I with other peroxidase substrates (12). It includes the rate of reduction of compound I to compound II by excess hydrogen peroxide (11). The rate constant of compound I reduction by melatonin is comparable to that by tyrosine (17), benzoic acid hydrazides (12) and nitrite (16), which suggests that melatonin could be an important electron donor for compound I even *in vivo*.

Recently, the reaction of two other indole derivatives with MPO has been investigated. It was shown that the rate constant between compound I and serotonin (5-hydroxytryptamine) or the amino acid tryptophan is (1.7 ± 0.1) × 10^7 M^{-1} s^{-1} (17) and 2.1 × 10^6 M^{-1} s^{-1} (18), respectively, demonstrating some effect of substituents on the indole ring oxidation by compound I (Table 1). Compared to chloride (11, 19), which is thought to be the physiological substrate of MPO, the reaction of compound I with melatonin is even about 2 orders of magnitude faster.

Since the pH inside phagocytizing cells is reported to be within the pH range 4.0–6.5 (20), the reaction between compound I and melatonin was also tested at pH 5. The apparent bimolecular rate constant was calculated to be (1.0 ± 0.08) × 10^7 M^{-1} s^{-1} at pH 5.0 and 25°C (intercept 14.8 ± 8.3 s^{-1}). Thus, compared with pH 7 the rate is slightly increased, which is in contrast to serotonin oxidation which was shown to be about 2.5 times slower at pH 5 compared with pH 7 (17). Compared with chloride oxidation by compound I at pH 5 (11), the rate constant of melatonin oxidation is about twice as high.

Reduction of compound II by melatonin. Melatonin also acts as one-electron donor for compound II. Figure 2A shows the direct conversion of compound II by 250 μM melatonin to the resting enzyme at pH 7. An isosbestic point at 442 nm is observed. At both 456 and 430 nm and high melatonin concentrations the time course was monophasic and exhibited a typical single-exponential behavior. At lower melatonin concentrations the reaction was too slow to guarantee pre-steady-state conditions and the enzyme cycled. An example is shown in Fig. 2B. A small steady-state phase is seen which, after depletion of hydrogen peroxide, is followed by an exponential decrease which strongly depended on melatonin concentration. But even in this concentration range (<750 μM melatonin) the observed pseudo-first-order rate constants are proportional to the concentration of melatonin (inset to Fig. 2B). The bimolecular rate constant determined at pH 7 is (9.6 ± 0.3) × 10^2 M^{-1} s^{-1} at 25°C. The small intercept (k_{off}) of (0.1 ± 0.02) s^{-1} demonstrates that there were no side reactions present.

Nevertheless, compound II reduction was also followed by conventional spectrophotometry as described

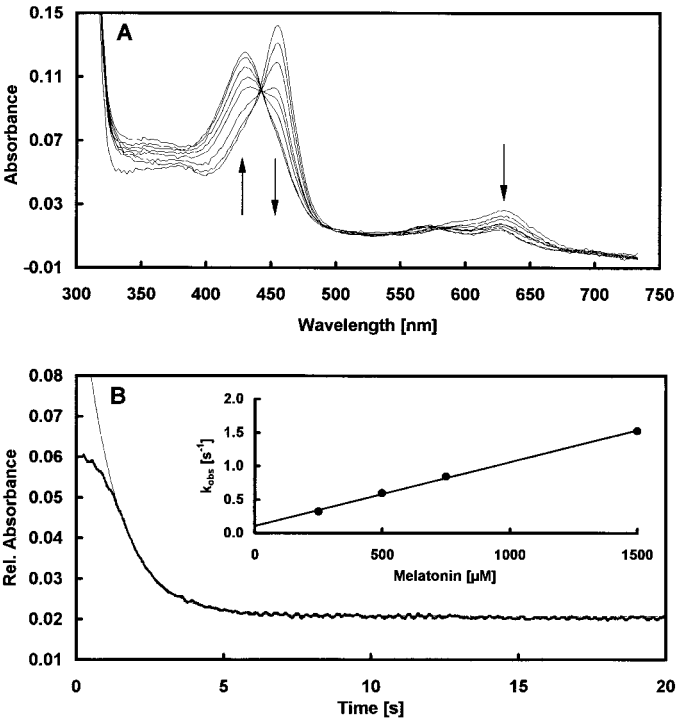


FIG. 2. Reaction of compound II with melatonin. (A) Spectral changes observed when 250 μM melatonin reacted with MPO compound II (2 μM heme). The first scan was taken 0.1 s after mixing, subsequent scans at 1.1, 2.1, 3.0, 3.5, 4.4, 6.2, and 8.3 s. Compound II was formed as described under Materials and Methods. The reaction was carried out in 100 mM phosphate buffer, pH 7. (B) Typical time trace of the reaction followed at 456 nm (750 μM melatonin). Final enzyme concentrations were 1 μM heme in 100 mM phospate buffer, pH 7. The single-exponential fit used for calculation of the pseudo-first-order rate constant is shown. Additionally, the inset shows pseudo-first-order rate constants for compound II reduction plotted against melatonin concentration.

by Kettle and Candaeis (18). Compound II was formed by adding an excess of hydrogen peroxide to ferric MPO. After addition of catalase to get rid of excess H_2O_2, melatonin was added and the resulting time trace was fitted to a single-exponential curve. Similar results as with the stopped-flow method were obtained within experimental error. The bimolecular rate at pH 5 is slightly enhanced [(2.2 ± 0.1) × 10^3 M^{-1} s^{-1} (intercept (0.2 ± 0.03) s^{-1})].

Comparing these data with the published rates of serotonin (17) and tryptophan (18) with compound II gives an important insight into the differences in reactivity of MPO compounds I and II. Table 1 clearly demonstrates that serotonin reacts with compound I about 10 times faster than tryptophan whereas the rate of serotonin with compound II is about 10^5 times higher than that of tryptophan. This unequivocally demonstrates that a pure thermodynamic consideration of this reaction is insufficient. Moreover, the one-electron redox potentials of the aromatic free radicals derived from the four compounds listed in Table 1 are

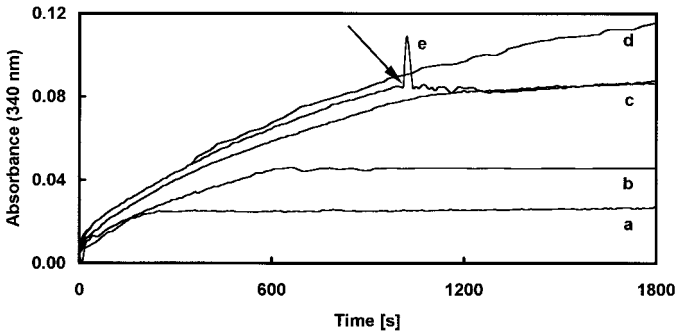


FIG. 3. The effect of hydrogen peroxide on melatonin (100 μM) oxidation mediated by myeloperoxidase (200 nM). The reactions were carried out in 100 mM phosphate buffer, pH 7, and followed at 340 nm. Reactions were started by addition of 10 (a), 20 (b), 50 (c), and 100 μM (d) H_2O_2. (e) The same setup as d with the exception that 10 mM NaCN was added during the reaction (arrow).

very similar (21–23). In contrast to compound I reduction, above all kinetic parameters (e.g., the rate of substrate binding) seem to determine the turnover of compound II. The structural requirements are much more restricted for the reaction of substrates with compound II than with compound I. Therefore, we suppose that there have to be important structural changes at the substrate binding site when MPO compound I is converted to compound II.

Melatonin oxidation by MPO. From the above results it is apparent that, although melatonin is a good substrate for compound I, it slowly reacts with compound II. To confirm this we also measured the ability of myeloperoxidase to catalyze the oxidation of melatonin. To monitor changes in the absorption spectrum of melatonin mediated by the MPO/H_2O_2 system, the spectral properties of both melatonin and the resulting metabolite were investigated. The spectrum of the resulting metabolite was similar to that of N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) as described recently (8, 10, 15). In contrast to melatonin, AFMK shows substantial absorbance in the range of 330–350 nm (not shown), which was the reason to follow the kinetics of melatonin oxidation at 340 nm.

Myeloperoxidase or hydrogen peroxide alone did not induce spectral changes of melatonin (not shown). This seems to be in contrast to the recently published paper of Tan *et al.* (13) who described that melatonin can scavenge hydrogen peroxide directly. However, in the H_2O_2 concentration range used in this paper (10–100 μM) no H_2O_2 mediated melatonin oxidation could be observed. On the contrary, with MPO in the presence of H_2O_2, melatonin was oxidized, albeit slowly. Inhibition of MPO by sodium cyanide (10 mM) quickly terminated melatonin oxidation (Fig. 3, curve e). Figure 3 demonstrates that the extent of melatonin oxidation was strongly dependent on the H_2O_2 concentration. Monitoring the spectral changes of the Soret band of MPO during melatonin oxidation showed unequivocally that the enzyme lingers at compound II (not shown). We have also tested the influence of superoxide dismutase on the rate and the extent of melatonin oxidation but no differences to the measurements without superoxide dismutase could be detected within experimental error. This underlines the transient-state results that melatonin is oxidized within the conventional peroxidase cycle and that reduction of compound II determines the overall reaction rate.

Influence of melatonin on monochlorodimedon chlorination. The chlorinating activity of MPO was inhibited dose-dependently by melatonin, at both pH 7 and pH 5 (Fig. 4A). Melatonin inhibited monochlorodimedone chlorination by 50% (IC_{50} value) at a concentration of 10 μM at pH 7 and 150 μM at pH 5 (Fig. 4B). These findings indicate that melatonin is a good inhibitor of the chlorination activity of MPO at pH 7. By virtue of its high rate of reduction of compound I, melatonin can compete with chloride and reversibly inhibit hypochlorous acid production. Since at pH 5 the bimolecular rate of chloride oxidation by compound I is

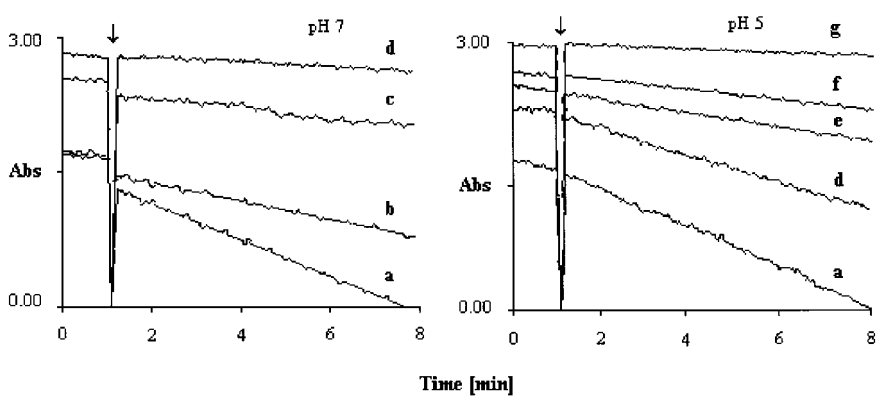


FIG. 4. Kinetics of monochlorodimedon (100 μM) chlorination by MPO (200 nM at pH 7.0 or 20 nM at pH 5.0), in the absence (a) or in the presence of melatonin (b–g) at pH 7 and 5. Melatonin in the assay was 10 (b), 50 (c), 100 (d), 150 (e), 200 (f), or 300 μM (g). The arrow points at the time of the enzyme addition. Assay conditions are reported under Materials and Methods.

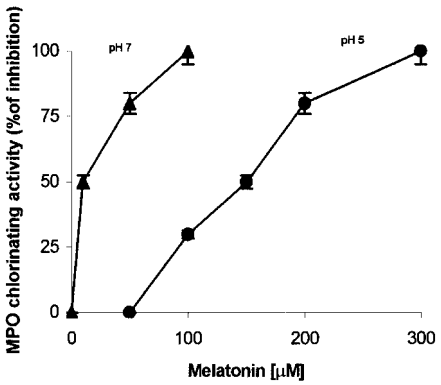


FIG. 5. Percentage of inhibition of the chlorinating activity by melatonin at pH 7.0 and pH 5.0. Data were obtained from the kinetic traces in Fig. 4.

about 2 orders of magnitude higher than at pH 7, at pH 5 and in the presence of chloride melatonin is less effective in formation of compound II, which is unable to oxidize chloride (Fig. 5).

CONCLUSIONS

Melatonin has been shown to have anti-inflammatory properties in a number of experimental set-ups (24–26). Most of its action has been reported as related to the scavenging of peroxynitrite. This is the first report showing that melatonin is an excellent reducing substrate for MPO compound I, at both pH 7 and 5. In accordance, it also effectively inhibits the chlorinating activity of the enzyme at pH 7, and, to a lesser extent, at pH 5. The fast rate of MPO compound I reaction with melatonin, as evaluated by the bimolecular rate constant we have measured, supports the conjecture that such a reaction could occur *in vivo.* On the other hand, melatonin is not such a good electron donor for MPO compound II, which would prevent a quick turnover of the enzyme, at least at the nanomolar physiological melatonin concentrations. However at sites of inflammation and infections, where the oxidative burst sustains high fluxes of reactive oxygen species, a local accumulation of melatonin may occur (27). Moreover, in the neutrophil several other electron donors like superoxide, tyrosine or ascorbate could act by reducing compound II back to the ferric enzyme and ensure that MPO is continuously turned over (18). So, in activated neutrophils MPO-mediated melatonin oxidation is likely to occur. This is supported by recent findings that neutrophils and monocytes possess receptors for melatonin (28, 29), that inflammatory cells can synthesize melatonin (30), and that it is involved in the regulation of the respiratory burst of human neutrophils (31).

The dramatic decrease of the rate constant of the reaction between compound II and melatonin may be indicative that important structural changes can occur at the substrate binding site when MPO compound I is converted to compound II. This could be suggestive for further research about structural features of the MPO binding site, as well as for the design of substrate analogues and inhibitors for myeloperoxidase.

ACKNOWLEDGMENTS

This work was supported by the Austrian Science Fund FWF (Grant P14187-MOB). One of the authors (M.A.) was sponsored by the European Science Foundation within the Reactor Project.

REFERENCES

1. Reiter, R. J. (1991) Pineal melatonin: Cell biology of its synthesis and its physiological interactions. *Endocr. Rev.* **12,** 151–180.
2. Hardeland, R., Balzer, I., Poeggeler, B., Uria, H., Behrmann, G., Wolf, R., Meyer, T. J., and Reiter, R. J. (1995) On the primary functions of melatonin in evolution: Mediation of photoperiodic signals in a unicell, photooxidation, and scavenging of free radicals. *J. Pineal Res.* **18,** 105–111.
3. Reiter, R. J., Tan, D.-X., Cabrera, J., D'Arpa, D., Sainz, R. M., Mayo, J. C., and Ramos, S. (1999) The oxidant/antioxidant network: Role of melatonin. *Biol. Signals Recept.* **8,** 56–63.
4. Tan, D.-X., Manchester, L. C., Reiter, R. J., Qi, W.-B., Zhang, M., Weintraub, S. T., Cabrera, J., Sainz, R. M., and Mayo, J. C. (1999) Identification of highly elevated levels of melatonin in bone marrow: Its origin and significance. *Biochim. Biophys. Acta* **1472,** 206–214.
5. Conti, A., Conconi, S., Hertens, E., Skwarlo-Sonta, K., Markowska, M., and Maestroni, G. J. M. (2000) Evidence for melatonin synthesis in mouse and human bone marrow cells. *J. Pineal Res.* **28,** 193–202.
6. Morrey, K. M., Mclachlan, J. A., Serkin, C. D., and Bakouche, O. (1994) Activation of human monocytes by the pineal hormone melatonin. *J. Immunol.* **153,** 2671–2680.
7. Garcia-Maurino, S., Gonzalez-Haba, M. G., Calvo, J. R., Rafii-El-Idrissi, M., Sanchez-Margalet, V., Goberna, R., and Guerrero, J. M. (1997) Melatonin enhances IL-2, IL-6 and IFN-gamma production by human circulating CD4+ cells: A possible nuclear receptor-mediated mechanism involving T helper type 1 lymphocytes and monocytes. *J. Immunol.* **159,** 574–581.
8. de Oliveira Silva, S., Ximenes, V. F., Catalani, L. H., and Campa, A. (2000) Myeloperoxidase-catalyzed oxidation of melatonin by activated neutrophils. *Biochem. Biophys. Res. Commun.* **279,** 657–662.
9. Klebanoff, S. J. (1991) Myeloperoxidase: Occurrence and biological function. *In* Peroxidases in Chemistry and Biology (Everse, J., Everse, K. E., and Grisham, M. B., Eds.), pp. 1–35, CRC Press, Boston.
10. Tesoriere, L., Avellone, G., Ceraulo, L., D'Arpa, D. Allegra, M., and Livrea, M. A. (2001) Oxidation of melatonin by oxoferry hemoglobin. A mechanistic study. *Free Radicals Res.*, in press.
11. Furtmüller, P. G., Burner, U., and Obinger, C. (1998) Reaction of myeloperoxidase compound I with chloride, bromide, iodide and thiocyanate. *Biochemistry* **37,** 17923–17930.
12. Burner, U., Obinger, C., Paumann, M., Furtmüller, P. G., and Kettle, A. J. (1999) Transient and steady-state kinetics of the oxidation of substituted benzoic acid hydrazides by myeloperoxidase. *J. Biol. Chem.* **274,** 9494–9502.
13. Tan, D.-X., Manchester, L. C., Reiter, R. J., Plummer, B. F., Limson, J., Weintraub, S. T., and Qui, W. (2000) Melatonin directly scavenges hydrogen peroxide: A potentially new meta-

bolic pathway of melatonin biotransformation. *Free Radical Biol. Med.* **29,** 1177–1185.

14. Hager, L. P., Morris, D. R., Brown, F. S., and Eberwein, H. (1966) Chloroperoxidase. II. Utilization of halogen anions. *J. Biol. Chem.* **241,** 1769–1777.
15. Marquez, L., and Dunford, H. B. (1995) Kinetics of reaction of tyrosine and dityrosine by myeloperoxidase compounds I and II. *J. Biol. Chem.* **270,** 30434–30440.
16. Burner, U., Furtmüller, P. G., Kettle, A. J., Koppenol, W. H., and Obinger, C. (2000) Mechanism of reaction of myeloperoxidase with nitrite. *J. Biol. Chem.* **275,** 20597–20601.
17. Dunford, H. B., and Hsuanyu, Y. (2000) Kinetics of oxidation of serotonin by myeloperoxidase compounds I and II. *Biochem. Cell Biol.* **77,** 449–457.
18. Kettle, A. J., and Candaeis, L. P. (2000) Oxidation of tryptophan by redox intermediates of myeloperoxidase and inhibition of hypochlorous acid production. *Redox Rep.* **5,** 179–184.
19. Furtmüller, P. G., Burner, U., Jantschko, W., Regelsberger, G., and Obinger, C. (2000) The reactivity of myeloperoxidase compound I formed with hypochlorous acid. *Redox Rep.* **5,** 173–178.
20. Jensen, M. S., and Bainton, D. F. (1973) Temporal changes in pH within the phagocytic vacuole of the polymorphonuclear neutrophilic leukocyte. *J. Cell Biol.* **56,** 379–388.
21. Harriman, A. (1987) Further comments on the redox potentials of tryptophan and tyrosine. *J. Phys. Chem.* **91,** 6102–6104.
22. Mahal, H. S., Sharma, H. S., and Mukherjee, T. (1999) Antioxidant properties of melatonin: A pulse radiolysis study. *Free Radicical Biol. Med.* **26,** 557–565.
23. Waltman, R. J., Diaz, A. F., and Bargon, J. (1984) Substituent effects in the electropolymerization of aromatic heterocyclic compounds. *J. Phys. Chem.* **88,** 4343–4346.
24. Cuzzocrea, S., Zingarelli, B., Gilad, E., Hake, P., Saltzman, A. L., and Szabò, C. (1997) Protective effect of melatonin in carrageenan-induced models of local inflammation: Relationship to its inhibitory effect on nitric oxide production and peroxinitrite scavenging activity. *J. Pineal Res.* **23,** 106–116.
25. Cuzzocrea, S., Zingarelli, B., Costantino, C., and Caputi, A. (1998) Protective effect of melatonin in a non-septic shock model induced by zymosan in the rat. *J. Pineal Res.* **25,** 24–33.
26. Costantino, G., Cuzzocrea, S., Mazzon, E., and Caputi, A. P. (1998) Protective effects of melatonin in zymosan-activated plasma-induced paw inflammation. *Eur. J. Pharmacol.* **363,** 57–63.
27. Tesoriere, L., D'Arpa, Conti, S., Giaccone, V., Pintaudi, A. M., and Livrea, M. A. (1999) Melatonin protects human red blood cells from oxidative hemolysis: New insights into the radical-scavenging activity. *J. Pineal Res.* **29,** 95–105.
28. Barjavel, M. J., Mamdough, Z., Raghbate, N., and Bakouche, O. (1998) Differential expression of the melatonin receptor in human monocytes. *J. Immunol.* **160,** 1191–1197.
29. Lopez-Gonzalez, M. A., Calvo, J. R., Segura, J. J., and Guerrero, J. M. (1993) Characterization of melatonin binding sites in human peripheral blood neutrophils. *Biotechnol. Ther.* **4,** 253–262.
30. Finocchiaro, L. M. E., Nahmod, V. E., and Launay, J. M. (1991) Melatonin biosynthesis and metabolism in peripheral-blood mononuclear leukocytes. *Biochem. J.* **280,** 727–731.
31. Pieri, C., Recchioni, R., Moroni, F., Marcheselli, F., Marra, M., Marinoni, S., and Di Primio, R. (1998) Melatonin regulates the respiratory burst of human neutrophils and their depolarization. *J. Pineal Res.* **24,** 43–49.