Vol. 147, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1987 Pages 354-360

EVIDENCE FOR THE ONE-ELECTRON OXIDATION OF 1-METHYL-4-PHENYL-2,3-DIHYDROPYRIDINIUM ($MPDP^+$)

W. Korytowski†, C. C. Felix, and B. Kalyanaraman*

National Biomedical ESR Center, Dept. of Radiology,
Medical College of Wisconsin, Milwaukee, WI 53226

Received July 13, 1987

SUMMARY: Optical data have shown that the neurotoxin metabolite 1-methyl-4-phenyl-2,3-dihydropyridinium undergoes one-electron oxidation/reduction in the presence of iron chelates. The activation energy for one-electron oxidation of 1-methyl-4-phenyl-2,3-dihydropyridinium is less than that for two-electron oxidation. Horseradish peroxidase catalyzes the oxidation of 1-methyl-4-phenyl-2,3-dihydropyridinium. Reactivity of 1-methyl-4-phenylpyridinyl radical is discussed in relation to the well-known pyridinyl radicals.

Studies dealing with the metabolism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are now an active area of research [1-5]. Interest in this area has grown primarily because of a recent discovery linking MPTP to the occurrence of Parkinsonian symptoms in users of an illicit narcotic contaminated with MPTP [6-8]. Subsequently, MPTP also has been shown to produce Parkinsonian-like symptoms in laboratory animals [9,10]. The pathological effects that are most consistently found following treatment of laboratory animals with MPTP appears to be selective destruction of dopaminergic neurons in the substantia nigra of the brain [9]. Recently it has been shown that biotransformation of MPTP to reactive metabolites, viz. 1-methyl-4-phenyl-2,3-dihydropyridinium ($MPDP^+$) and 1-methyl-4-phenylpyridinium (MPP^+), species by brain monamine oxidase-B (MAO-B) is crucial for the expression of neurotoxicity of MPTP [11,12].

†On leave from the Department of Biophysics, Institute of Molecular Biology, Jagiellonian University, Krakow, Poland.

*To whom correspondence should be addressed.

Abbreviations: MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; $MPDP^+$: 1-methyl-4-phenyl-2,3-dihydropyridinium; MPP^+: 1-methyl-4-phenylpyridinium; MPP: 1-methyl-4-phenylpyridinyl radical; MAO-B: monamine oxidase-B; EDTA: ethylenediaminetetraacetic acid; HRP: horseradish peroxidase.

0006-291X/87 $1.50

There is now a widespread belief among investigators in this area that toxic oxy-radical intermediates are formed during bis biotransformation of MPTP [13-20]. The metabolite MPP^+ is structurally related to the bipyridinium pesticide paraquat, and by analogy with known redox chemistry of paraquat, MPP^+ was proposed to undergo one-electron reduction to form MPP·, with subsequent formation of superoxide and hydrogen peroxide as a result of reoxidation of MPP· by molecular oxygen [13,14]. However, this hypothesis has not been supported by data. Neither MPP· nor superoxide radicals have been reported during chemical and enzymatic oxidation of MPTP or its metabolites and, in a recent comparison of the ability of paraquat and MPP^+ to undergo redox activation by rat liver microsomes and NADPH, the extent of one-electron reduction of MPP^+ was found to be negligible [21]. These findings are in keeping with the extremely negative redox potential for MPP^+ [22], as compared to paraquat.

The second major metabolite $MPDP^+$ has been suggested to undergo spontaneous disproportionation to MPP^+ and MPTP at physiological pH values [2]. Recently, this decomposition reaction has been shown to be catalyzed by metal chelates [17,23].

In this communication, we present evidence for (i) the one-electron oxidation of $MPDP^+$ in the presence of iron-chelates, and (ii) the oxidation of $MPDP^+$ by the horseradish peroxidase (HRP)/H_2O_2 system. The structure and reactivity of the one-electron oxidation product MPP· are discussed.

MATERIALS AND METHODS

$MPDP^+$ perchlorate was obtained from Research Biochemicals Inc. Catalase (40,000 Sigma units/mg protein) and superoxide dismutase (from bovine blood) were obtained from Sigma Chemical Company. Ferric chloride (Sigma Chemical Company) and 1,10-phenanthroline (Aldrich Chemical Copmpany) were used as received.

HRP (Type VI) was purchased from Sigma Chemical Company. The enzyme was denatured by heating in boiling phosphate bufer (pH 8.0, 100 mM) for 30-40 min. [24]. Optical absorbance measurements were made using a Perkin-Elmer 320 spectrophotometer. Initial rates of formation and depletion of MPP^+ and $MPDP^+$ were monitored using the following parameters: MPP^+: λ_{max} = 290 nm, ϵ = 18,000 $M^{-1}cm^{-1}$; $MPDP^+$: λ_{max} = 345 nm, ϵ = 17,400 M^{-1} cm^{-1} [25]. The iron-EDTA complex was added as a pre-formed complex prepared by adding one equivalent of Fe^{3+} to two equivalents of EDTA in acidic media. The production of Fe^{2+}-phenanthroline complex was monitored optically at 510 nm. The amount for Fe^{2+} released was calculated using ϵ = 11,500 M^{-1} cm^{-1} for the Fe^{2+}-phenanthroline complex [26].

RESULTS

Upon anerobic incubation of $MPDP^+$ and Fe(III)-EDTA in the presence of ortho--phenanthroline, we noticed a decrease in absorbance at 345 nm and an increase in absorbance at 510 nm (Fig. 1) consistent with oxidation of $MPDP^+$ and formation of Fe^{2+}. Analysis of time-dependent changes (Fig. 2) in the optical absorption spectrum indicated that the initial rate of depletion of $MPDP^+$ approximately equals the initial rate of production of Fe(II)-phenanthroline complex ($-d[MPDP^+]/$ = 0.46 ± 0.02 μM/min; d[Fe(II)-phenanthroline]/dt = 0.38 ± 0.02 μM/min). Thus the stoichiometry between $MPDP^+$ disappearance and Fe(II)-phenanthroline production is estimated to be ca. 1:0.8 for up to 60 minutes [Fig. 2]. After this time production of Fe(II)-phenanthroline plateaus even though decomposition of $MPDP^+$ continues to occur (Fig. 2).

The rate of formation of Fe(II)-phenanthroline complex increased linearly with increasing concentration of Fe(III) chelate until an approximate 1:1 stoichiometry between $MPDP^+$ and Fe(II)-EDTA was reached (Fig. 3). These results

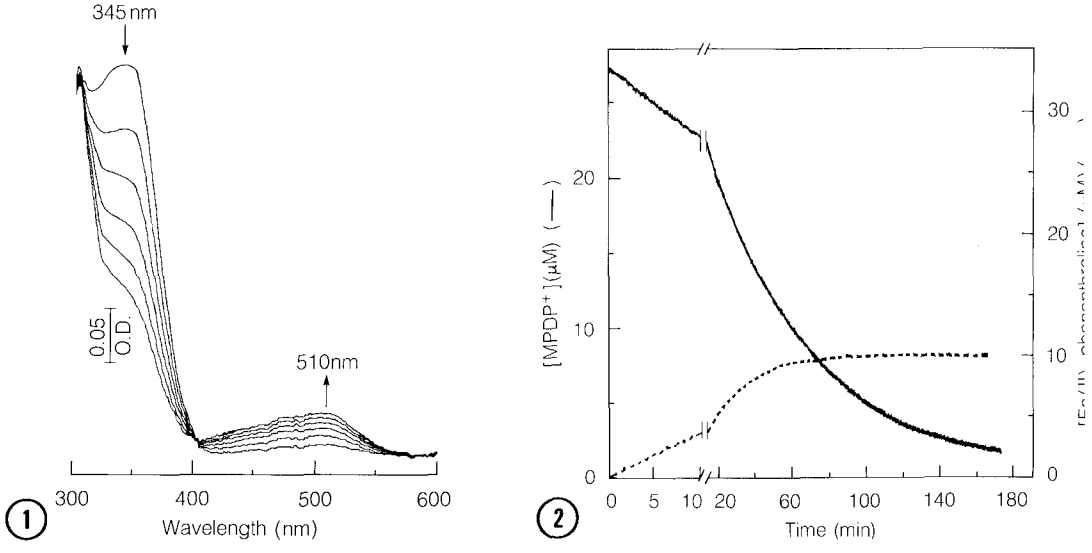
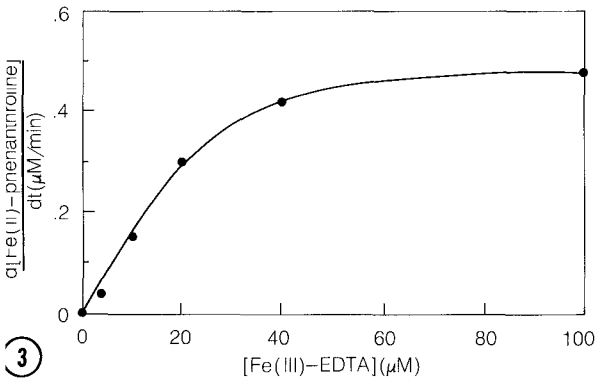


Figure 1. Optical changes occurring during the oxidation of $MPDP^+$ by Fe(III)-EDTA. The oxidation was carried out anerobically in a phosphate bufer (pH 8.0) at 37°C containing 30 μM $MPDP^+$, 50 μM Fe(III)-EDTA, and 2 mM 1,10-phenanthroline. The maxima at 345 and 510 nm belong to $MPDP^+$ and Fe(II)-phenanthroline, respectively.

Figure 2. The stoichiometric relationship between the rate of depletion of $MPDP^+$ and the rate of production of Fe(II)-phenanthroline complex under conditions identical to Fig. 1. Depletion of $MPDP^+$ and formation of Fe(II)-phenanthroline complex were monitored at 345 and 510 nm, respectively.



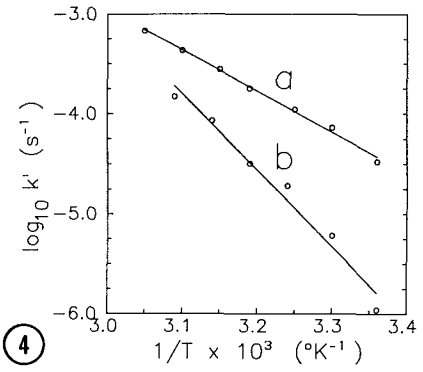


Figure 3. The rate of production of Fe(II)-phenanthroline complex as a function of Fe(III)-EDTA concentrations during the oxidation of 30 μM $MPDP^+$ in a phosphate buffer (pH 8.0) at 37°C containing 2 mM 1,10-phenanthroline.

Figure 4. Variation of reaction rate with temperature: a, oxidation of 30 μM $MPDP^+$ in a phosphate buffer (pH 8.0, 100 mM) containing 10 μM Fe(III)-EDTA, and b, oxidation of 30 μM $MPDP^+$ in a phosphate buffer (pH 8.0, 100 mM) containing 30 μM desferrioxamine. The rate of reaction was monitored at 345 nm.

are consistent with a one-electron oxidation/reduction mechanism for oxidation of $MPDP^+$ by iron chelates.

We monitored the rate of depletion of $MPDP^+$, with and without the added iron-chelates, as a function of temperature. In Fig. 4 are shown Arrhenius plots of the apparent first order rate constant, k', vs. 1/T. From these linear plots, the energy activation, E_a, for decomposition of $MPDP^+$ in the presence and in the absence of Fe(III)-EDTA was calculated. We obtained E_a for the $MPDP^+$ decomposition (in the absence of Fe(III)-EDTA) to be 36,640 cal $mole^{-1}$ and for the iron-EDTA catalyzed reaction to be 18,320 cal $mole^{-1}$. Thus, the addition of Fe(III)-EDTA chelates lowers the activation energy for the oxidation; one-electron oxidation of $MPDP^+$ (to a free radical intermediate MPP·) is energetically favored over the two-electron oxidation under the present experimental conditions.

Since most peroxidatic oxidations also involve free radical intermediates [27], we studied the oxidation of $MPDP^+$ by HRP. We found that addition of HRP to an aerobic solution of $MPDP^+$ enhanced its oxidation by a factor of five or so (Fig. 5). Note that extraneous addition of hydrogen peroxide was not necessary, since autoxidation of $MPDP^+$ alone generates hydrogen peroxide [23]. Addition of catalase inhibits the HRP-catalyzed oxidation of $MPDP^+$ (Fig. 5). Heat-denatured HRP did not enhance the autoxidation of $MPDP^+$.

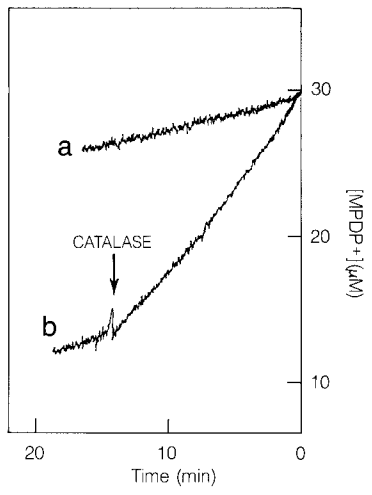


Figure 5. Horseradish peroxidase-catalyzed oxidation of $MPDP^+$. a, control using 30 μM $MPDP^+$ and b, 30 μM $MPDP^+$ plus 10 μg/mL HRP. The oxidation was carried out in a phosphate bufer (pH 8.0) in air. Catalase (5 μg/mL) was added at the point marked ↑.

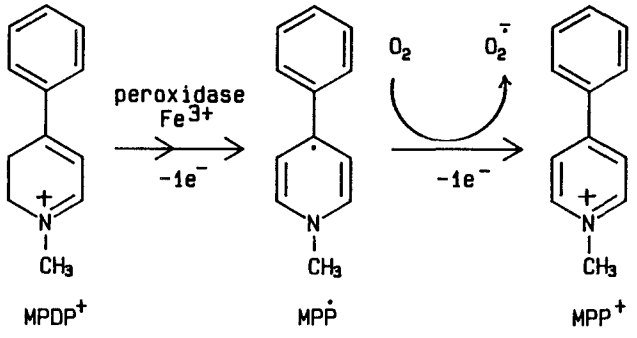
DISCUSSION

MPP· has been proposed as a key toxic free radical intermediate in the metabolism of MPTP [14]. Our data suggest that one-electron oxidation of $MPDP^+$ can occur, whereas one-electron reduction of MPP^+ seems unlikely based on reduction potentials and recent data [22,28].

The enzymes responsible for the production of radicals from MPP^+, if this indeed occurs, are not known. The substantia nigra, the locus of MPTP toxicity, reportedly contains high levels of metal ions [29,30]. This nucleus also exhibits high peroxidase activity which is decreased in Parkinson's disease [30]. Since most peroxidatic oxidations involve radical intermediates, the production of MPP· radicals via oxidation of $MPDP^+$ seems likely. It is conceivable that the mammalian peroxidase prostaglandin hydroperoxidase that is present in the brain also can oxidize $MPDP^+$ to MPP·.

The MPP· radical, like paraquat and $NAD^{\cdot}$ radicals, is a pyridinyl radical [31], i.e. species formed during one-electron reduction of pyridinium ion or one-electron oxidation of dihydropyridine. Pyridinyl radicals react fairly rapidly with oxygen [31]. Consistent with this MPP· generated from pulse radiolysis of MPP^+ has been shown to react rapidly with oxygen [32]. Unlike

paraquat radical which is mono-positively charged, MPP· is a neutral radical. In this respect, MPP· resembles NAD·. Neutral pyridinyl radicals such as NAD·, have previously been shown to react with molecular oxygen to form superoxide [33]. Thus the Scheme shown below for the oxidation of $MPDP^+$ to MPP^+ appears plausible. In the absence of oxygen, MPP·, like PQ^{+}· and NAD·, probably decays via disproportionation or dimerization.



Scheme. One-electron oxidation of $MPDP^+$

Most pyridinyl radicals have hitherto been observed only by pulse radiolysis. A search for identification of the radical metabolite MPP· by electron spin resonance is in progress.

ACKNOWLEDGEMENTS

This work was supported by National Institutes of Health Grants GM-29035, RR-01008 and the Polish Ministry of Science RP II, 13.2.11.

REFERENCES

1. Chiba, K., Trevor, A., and Castagnoli, Jr., N. (1984) Biochem. Biophys. Res. Commun. 120, 574-578.
2. Chiba, K., Peterson, L. A., Castagnoli, K. P., Trevor, A. J. and Castagnoli, Jr., N. (1985) Drug. Metab. Dispos. 13, 342-347.
3. Weissman, J., Trevor, A. J ., Chiba, K., Peterson, L. A., Caldera, P., Castagnoli, Jr., N. an Ballie, T. (1985) J. Med. Chem. 28, 997-1001.
4. Salach, J. I., Singer, T. P., Castagnoli, J., N., and Trevor, A. J. (1984) Biochem. Biophys. Res. Commun. 125, 831-835.
5. Cashman, J. R. and Ziegler, D. M. (1986) Mol. Pharmacol. 29, 163-167.
6. Davis, G. C., Williams, a. C., Markey, S. P., Ebert, M. H., Caine, E. D., Reichert, C. M. and Kopin, I. J. (1979) Psychiatr. Res. 1, 249-254.
7. Langston, J. W., Ballard, P., Tetrvd, J. W., and Irwin, I. (1983) Science 219, 979-980.
8. Wright, J. M., Wall, R. A., Perry, T. L. and Paty, D. W. (1984) N. Engl. J. Med. 310, 325- .

9. Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H. E., Jacobowitz, D. M. and Kopin, I. J. 91984) Proc. Natl. Acad. Sci. USA 80, 4546-4550.
10. Langston, J. W., Forno, L. S., Rebert, C. S. and Irwin, I. (1984) Brain Res. 292, 390-394.
11. Heikkila, R. E., Manzino, L., Cabbat, F. S. and Duvoisin, R. S. (1984) Nature (Lond.) 311, 467-469.
12. Langston, J. W., Irwon, I., and Langston, E. B. (1984) Science 225, 1480-1482.
13. Lewin, R. (1984) Science 225, 1460-1462.
14. Johannessen, J. M., Chiueh, C. C., Burns, R. S. and Markey, S. P. (1985) Life Sci. 36, 219-224.
15. Cohen, G. and Mytilineou, C. (1985) Life Sci. 35, 237-242.
16. Cohen, G. (1984) Neurotoxicology 5, 77-82.
17. Poirier, J. and Barbeau, A. (1985) Bichem. Biophys. Res. Commun. 131, 1284-1289.
18. Poirier, J., Donaldson, J. and Barbeau, A. (1985) Biochem. Biophys. Res. Commun. 128, 25-33.
19. Corsini, G. V., Pintus, S., Chiueh, C. C., Weiss, J. F. and Kopin, I. J. (1985) Eur. J. Pharmacol. 119, 127-128.
20. Young, V. W., Perry, T. L., and Krisman, A. A. (1986) Neurosci. Lett. 63, 56-60.
21. Sinha, B. K., Singh, Y., and Krishna, G. (1986) Biochem. Biophys. Res. Commun. 135, 583-588.
22. Ames, J. R., Castagnoli, Jr., N., Ryan, M. D. and Kovacic, P. (1986) Free Rad. Res. Commun. 2, 107-113.
23. Korytowski, W., Felix, C. C. and Kalyanaraman, B. (1987) Biochem. Biophys. Res. Commun. 144, 692-698.
24. Kalyanaraman, B., Mason, R. P., and Sivarajah, K. (1982) Biochem. Biophys. Res. Commun. 105, 217-224.
25. Wu, E. Y., Chiba, K., Trevor, A. J. and Castagnoli Jr., N., (1986) Life Sci. 39, 1695-1700.
26. Shinar, E., Navok, T. and Chevion, M. (1983) J. Biol. Chem. 258, 14778-14783.
27. Yamazaki, I. (1977) in Free Radicals in Biology (W. A. Pryor, ed.) Vol. III, Academic Press, N. Y., pp. 183-218.
28. Trever, A. J., Castagnoli Jr., N., Caldera, P., Ramsay, R. R. and Singer, T. P. (1987) Life Sci. 40, 713-719.
29. Hill, J. M. and Switzer, R. C. (1984) Neurosci. 11, 595-603.
30. Ambani, L. M., Van Woert, M. H. and Murphy, S. (1975) Arch. Neurol. 32, 114-118.
31. Kosower, E. M. (1976) in Free Radicals in Biology (W. A. Pryor, ed.) Vol. II, Academic Press, N. Y., pp. 1-53.
32. Chacon, J. W., Chedekel, M. R., Land, E. J. and Truscott, T. G. (1987) Biochem. Biophys. Res. Commun. 144, 957-964.
33. Land, E. J. and Swallow, A. J. (1968) Biochim. Biophys. Acta 162, 327-337.