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OXYGEN ACTIVATION

III. THE ROLE OF MONOPROTONATED *p*-PHENYLENEDIAMINES*,**

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SUMMARY

1. The monoprotonated form of *p*-phenylenediamines catalyzes the autoxidation of the conjugated base and of other substrates by activating the O_2 molecule.

2. The main inferences are: (i) in studies of electron transport mediated by *p*-phenylenediamines the substrate oxidation may in part bypass the respiratory chain, whereby lower P:O ratios will result; and (ii) tetrahydropteridines may participate in O_2 activation.

3. The possibility is discussed of O_2 activation in biological systems by various species, other than metallic cations and their complexes.

INTRODUCTION

During our studies of O_2 activation[1–3] it was observed that the rate of autoxidation of *p*-phenylenediamines, as judged by semiquinone formation, is maximal at a certain pH value. A search of the literature indicated that LU VALLE, GLASS AND WEISSBERGER[4] had observed the same effect by determining the rate of O_2 uptake as a function of the pH; yet those investigators stated that a definite discussion of the phenomenon was not possible with the available information.

It is shown here that the observed pH dependence of *p*-phenylenediamine autoxidation is to be ascribed to catalysis by the monoprotonated form of the diamine very probably by way of O_2 activation. In view of the increasing number of studies on biological electron transport employing *p*-phenylenediamines, and especially because a similar diamine structure appears in the tetrahydropteridine nucleus, the observed O_2 activation is of significance in biochemistry.

EXPERIMENTAL

All the pertinent information is to be found in the earlier papers of the series[1,2]. The p*K* for *p*-phenylenediamine was determined potentiometrically.

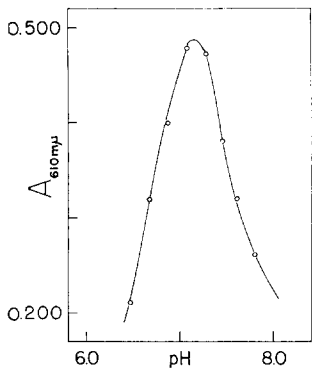
* Part II, ref. 2.

** The work reported in this and other papers of this series is taken from a thesis to be submitted by K. ZINNER to the Universidade de São Paulo, in partial fulfilment of the requirements for the degree of Doctor of Science.

RESULTS AND DISCUSSION

When the autoxidation of TMPD is studied in 0.01 M or 0.1 M phosphate buffers or in 0.01 M veronal buffers of various pH, the initial velocity in terms of the formation of the very stable semiquinone (Wurster blue radical)[5] shows a sharp maximum at pH 7.1 (Fig. 1).

The autoxidation of *p*-phenylenediamine was studied by adding various amounts of HCl to the diamine in methanol–water (3:1; v/v) mixtures, a solvent in which the radical cation formed is reasonably stable[5]. Here a poorly pronounced maximum was observed just above pH 7.0 (Fig. 2).



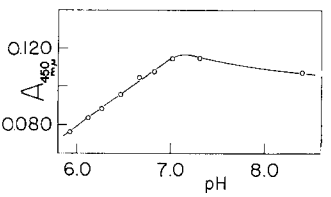


Fig. 1. Velocity of autoxidation of a 0.55 mM solution of TMPD in 0.1 M phosphate buffer as a function of the pH. The ordinate represents the absorption of the blue semiquinone developed at 610 mμ in 500 sec.

Fig. 2. Velocity of autoxidation of a 0.05 M solution of *p*-phenylenediamine in methanol–water (3:1; v/v) containing various amounts of HCl. Abcissa: the apparent pH. Ordinate: the semiquinone absorption developed at 450 mμ in 1500 sec.

In the direction of increasing pH, the ascending part of the maximum is due to liberation of the free base. The descending part might be due to a decrease in the concentration of the semiquinone[4]; this possibility was disposed of by observing that in anaerobic conditions the absorption spectrum of the Wurster blue radical was not affected by raising the pH from 6.8 to 8.6 with Na_3PO_4.

The descending part of the maximum may be ascribed to the disappearance of a catalytic species, presumably the monoprotonated diamine. This is also indicated by the behaviour of the mixed system *p*-phenylenediamine–TMPD: it can be seen (Fig. 3) that TMPD in its monoprotonated form in a concentration as low as 10 μM greatly increases the formation of the radical cation from *p*-phenylenediamine.

Since it is hardly conceivable that $TMPDH^+$ participates directly in electron transport, it must activate either the free base or O_2. Despite the presence of a positive charge the cation should display electron donor ability (in the sense of MULLIKEN[6]) somewhat similar to that of anilines. Therefore, if the interaction is of the charge-transfer type, the cation will associate with the electron acceptor O_2 molecule[7]. The great efficiency of $TMPDH^+$ as an activator compared with that of TMPD would then be due to the co-operation of an out-of-plane hydrogen.

The outstanding O_2 activation by $TMPDH^+$ raises the possibility that in

studies of biological electron transport with *p*-phenylenediamines as mediators between the substrate and the respiratory chain, part of the substrate oxidation bypasses the respiration–phosphorylation chain. This may be a contributing factor to the lowering of the P:O ratio observed as a result of increased TMPD concentrations[8–10]. Care should therefore be taken in employing *p*-phenylenediamines in studies of electron transport, especially if the pH of the maximal rate of autoxidation is close to the first pK_a (*cf.* APPENDIX).

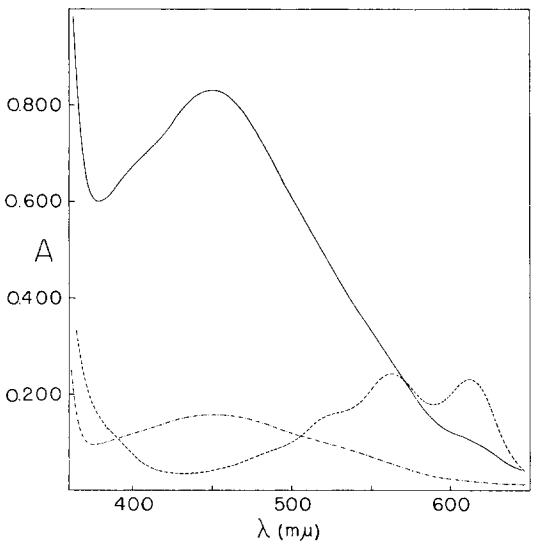


Fig. 3. The catalytic effect of $TMPDH^+$ upon the autoxidation of *p*-phenylenediamine. Absorption spectra: –.–.–.–, 42.8 mM *p*-phenylenediamine (pH 8.49) after 3410 sec; – – – –, 5.44 mM TMPD (pH 9.13) after 3040 sec; ———, 42.8 mM *p*-phenylenediamine + 5.44 mM TMPD (pH 9.07) after 2700 sec. Solvent: methanol–water (3:1; v/v). The differences in pH between the mixture and controls are responsible for only an insignificant part of the effect. Only a small part of the *p*-phenylenediamine semiquinone may have been formed by electron exchange between *p*-phenylenediamine and the Wurster blue radical cation as ε_{max} of the semiquinone from *p*-phenylenediamine is about half that from TMPD (ref. 5).

Because a *p*-phenylenediamine-like structure is part of the tetrahydropteridine nucleus, it is conceivable that the latter—being indeed an excellent donor[11]—could participate in O_2 activation. From data of MAGER AND BERENDS[12] it is evident that the initial rate of O_2 uptake in a system of a tetrahydropteridine and NADPH or cysteine is somewhat higher than expected on the basis of the cyclic role of the tetrahydropteridine. It may well be that a fraction of NADPH and of cysteine is directly oxidized by the tetrahydropteridine–O_2 complex.

The results presented in this and other papers[1–3] show that O_2 activation may be a more general phenomenon than is normally suspected. Thus besides the well-known effect of metallic cations and of their complexes, we have shown that activation can also be brought about by organic cations and anions[1,2] and by an inorganic ion, the iodide ion[3]. These activating species are natural in origin or related to natural compounds, and the possibility that in biological systems O_2 might be activated by such compounds was pointed out by HAYAISHI[13]. For the monooxygenase reactions HAYAISHI[13] suggested that if the metal cation does not participate in the reaction,

the reductant could fulfil the role of the metal, a phenomenon that would be both important and interesting.

MASON[14] has pointed out the convenience of analyzing donor–acceptor interactions of O_2 in connection with oxidase function.

APPENDIX

If we assume that both the catalyzed and uncatalyzed autoxidation of phenylenediamines are first order in the base and of the same order in oxygen, and that the catalyzed reaction is first order in the catalyst, then the initial velocity of radical formation will be given by

$$v = \frac{d(R^{\bullet})}{dt} = k(B_0 - BH^+)pO_2^n + k_{cat}(B_0 - BH^+)(BH^+)pO_2^n$$

where B_0 is the initial concentration of the diamine and BH^+ the concentration of catalyst cation.

The initial velocity will be maximal when

$$\frac{dv}{d(BH^+)} = -k + k_{cat}(B_0) - 2k_{cat}(BH^+) = 0$$

that is when

$$\frac{BH^+}{B_0} = \frac{1}{2} - \frac{k}{2B_0k_{cat}}$$

Hence if $2B_0k_{cat} \gg k$, maximal initial velocity will be attained when pH = pK. The closest situation occurs with TMPD (pH 6.4 ref. 15; pH_{max} 7.1). For *p*-phenylenediamine we determined the pK and found it to be 1.1 pH units lower than the pH_{max} which is 7.1. For other N-methylated derivatives of *p*-phenylenediamine the maximum—as judged from the curves of O_2 uptake[4]—seems even more removed from the pK value.

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