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Catalysis by heteropentalenes of electron transfer to oxygen from ferredoxin-NADP$^+$ oxidoreductase reduced by NADPH

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A range of heteropentalene and bipyridinium compounds have been tested as catalysts of electron transfer to oxygen from spinach ferredoxin-NADP$^+$ oxidoreductase reduced by NADPH. For a particular class of compound, the rate of oxygen reduction increased with increasing midpoint potential of the compound under conditions in which reduction of the compound was rate-limiting. Compounds with similar midpoint potentials from different structural classes showed marked differences in rate, attributed to specificity in the interaction with ferredoxin-NADP$^+$ oxidoreductase.

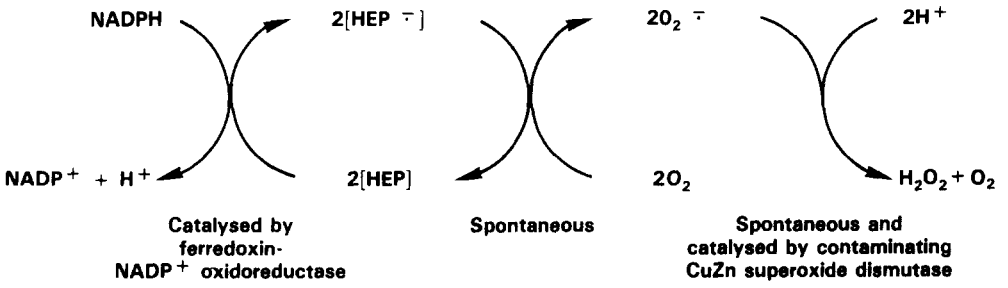
The light-driven reduction of oxygen by chloroplasts occurs primarily through electron transfer from ferredoxin reduced by Photosystem I [1,2]. In intact chloroplasts, the principal oxidant for ferredoxin is $NADP^+$, in a reaction catalysed by ferredoxin-$NADP^+$ oxidoreductase. In a chloroplast-free system, ferredoxin-$NADP^+$ oxidoreductase can catalyse the reverse reaction, i.e., the oxidation of NADPH by ferredoxin, which is subsequently reoxidised by oxygen [3].

We have recently reported the electrochemical and herbicidal properties of a number of heteropentalenes [4,5]. In broken chloroplasts, these compounds are found to act as Photosystem I electron acceptors at a concentration of about 10^{-6} M, which is of the same order of magnitude as that for the bipyridinium herbicide, paraquat.

The reduction potentials of the dioxathiadiaza-heteropentalenes range from about -225 to -415 mV. Since the reduction potentials of the O_2/O_2^- couple [6] and the $NADP^+/NADPH$ couple [7] are reported to be around -155 and -330 mV, respectively, at pH 7.5, it should be thermodynamically possible for these heteropentalenes to catalyse oxygen reduction by the sequence of reactions in Scheme I, where the heteropentalene (HEP) molecules are taking the place of ferredoxin in the natural system. Such a reaction has already been demonstrated using bipyridinium compounds such as paraquat [8]. Whereas ferredoxin further reduces superoxide to hydrogen peroxide [1,2], this reaction does not occur in the case of the heteropentalenes (O'Neil, P., Camilleri, P. and Bowyer, J.R., unpublished results).

Heteropentalene-catalysed oxygen uptake was measured at 20°C using a Clark-type oxygen electrode in the presence of NADPH and spinach ferredoxin-$NADP^+$ oxidoreductase. Full details are described in a footnote to Table I. Heteropentalenes were added as solutions in ethanol. Control experiments indicated that no oxygen uptake was

Abbreviation: DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide.



Schema 1

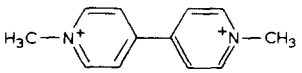
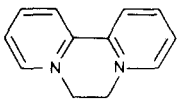
observed in the absence of reductase or NADPH, and the rate in the absence of added heteropentalene was negligible. Ethanol at the concentration present (0.1% v/v) for heteropentalene additions had no effect on the rate with paraquat. Spin-trapping experiments using 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) to detect superoxide and hydroxyl radicals were carried out essentially as described in Ref. 9. NADPH and ferredoxin-NADP$^+$ oxidoreductase were obtained from Sigma Chemical Co. Ltd. and DMPO from Aldrich.

Varying the concentrations of NADPH, ferrodoxin-NADP$^+$ oxidoreductase and acceptor indicated that the concentrations of the latter two limited the initial rate of oxygen uptake. Results are collected in Table I which also shows data obtained for the bipyridinium compounds, paraquat and diquat, and for a novel class of

TABLE I

EFFECTS OF HETEREOPENTALENES, PARAQUAT AND DIQUAT ON OXYGEN REDUCTION BY NADPH IN THE PRESENCE OF FERREDOXIN-NADP$^+$ OXIDOREDUCTASE

E_7^1 has been determined by pulse radiolysis in pH 7 buffer (Ref. 4). $E_{p/2}$ has been determined by cyclic voltammetry in methanol/water (3:1, v/v) (Refs. 5 and 10). HEP, heteropentalene.

Compound	Structure	E_7^1 (mV)	$E_{p/2}$ (mV)	Initial rate of oxygen uptake (μmol O_2 per mg protein per h)	
				on addition of 10^{-5} M compound compound	on addition of 10^{-4} M compound compound
HEP I	R=H, X=CH_2, Y=O	−416	−470	2.7	13.6
HEP II	R=H, X=S, Y=O	−375	−420	6.7	41.9
HEP III	=H, X=SO, Y=O	−277	−350	8.2	51.2
HEP IV	R=H, X=SO_2, Y=O	−227	−300	17.3	147.6
HEP V	R=CH_3, X=CH_2, Y=S		−400	36.0	214.1
HEP VI	R=H, X=S, Y=S		−250	82.9	414.3
Paraquat		−477		10.6	47.9
Diquat		−350		13.5	88.4

heteropentalenes [10], denoted by heteropentalene V and heteropentalene VI. It is of interest to note from the table that in the three classes of compounds a more positive reduction potential leads to an enhanced rate of oxygen uptake; thus a molecule like heteropentalene IV is more efficient in reducing oxygen than heteropentalene I. This is also true comparing paraquat to diquat and heteropentalene V to heteropentalene VI. This is in contrast to the relationship between reduction potential and rate of reaction with oxygen in which a more positive reduction potential is correlated with decreasing rate, and confirms that the rate-limiting step is associated with the reduction of heteropentalene. Table I also shows that the novel class of heteropentalenes (as represented by heteropentalene V and heteropentalene VI) are capable of catalysing reduction of oxygen much more efficiently than dioxathiadiaza heteropentalenes or bipyridinium compounds with similar reduction potentials (for example, compare heteropentalene II and heteropentalene V). This might indicate that a certain degree of specificity is involved in the interaction of these redox compounds with the ferredoxin-$NADP^+$ oxidoreductase, possibly at the ferredoxin-binding site.

In contrast to experiments using the heteropentalenes reduced by illuminated chloroplasts [4,9], superoxide was not detected using the spin trap DMPO unless KCN was present (at 2 mM). This indicates that CuZn superoxide dismutase was present as a contaminant in the ferredoxin-$NADP^+$ oxidoreductase [1]. Added KCN or superoxide dismutase had minor effects on the rate of oxygen uptake, whereas added catalase (16000 units/ml) more than halved the rate (for example, from 81.9 to 29.8 μmol O_2 per mg protein per h with 10^{-5} M heteropentalene VI as acceptor) and caused the release of oxygen when added during the assay. This is consistent with the principal product of oxygen reduction being hydrogen peroxide resulting from superoxide dismutation.

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