A Manganese Cluster Bound to a Bilayer Membrane: a Chemical Model for the Oxygen-forming Centre of Photosynthesis

Natalia P. Luneva, Eugenia I. Knerelman, Vladimir Ya. Shafirovich, and Alexander E. Shilov

U.S.S.R. Academy of Sciences Institute of Chemical Physics, Chernogolovka, 142 432 U.S.S.R.

Manganese(IV) bound to lipid vesicles is an active catalyst for dioxygen evolution in the presence of one-electron oxidants, such as $[Ru(bpy)_3]^{3+}$ and $[Fe(bpy)_3]^{3+}$ (bpy = 2,2'-bipyridyl).

Manganese is known to play an important role in O_2 evolution in photosystem II of green plants (PSII).[1,2] Because of its instability, the Mn-containing enzyme has not yet been isolated. The number of Mn atoms in the active centre as well as their oxidation state in the active form of the catalyst is still a matter of controversy.[3—5] Investigation of chemical systems modelling the dioxygen-forming centre may help in understanding this important process.

Earlier we have shown[6] that MnO_2 is the catalyst of O_2 formation under the action of the one-electron oxidant $[Ru(bpy)_3]^{3+}$, the redox potential of which (1.26 V)[7] is close to that of the chlorophyll cation radical $P_{680}^{+\bullet}$ (1.12 V).[8] To develop this catalytic system and to model it more closely on the oxygen-forming centre of photosynthesis, it was essential to find out whether the catalytic properties of Mn^{IV} would be preserved when it was incorporated in an organic membrane (a high-valent Mn compound might in principle oxidize the organic membrane instead of catalysing O_2 evolution).

The catalyst was prepared by ultrasonic dispersion (30 min) of dipalmitoyl-DL-α-phosphatidylcholine (DPPC) (9.2 mg) in 2.5 ml of 0.002 M-$MnSO_4 \cdot H_2O$ in 0.04 M-borate buffer (pH 8.3). The vesicles were subsequently separated by gel-filtration through Sephadex G-150. Gel-chromatography analysis on Sepharose 2B, coupled with atomic absorption analysis for Mn, showed that the catalyst contains unilamellar vesicles with strongly bound manganese (see Figure 1). Irreversible binding proceeds only with the oxidized form of manganese, which is produced in the reaction of Mn^{II} with air during dispersion. The oxidation state of manganese in the catalyst, determined by measuring the amount of CO_2 evolved in the reaction of the oxidized Mn with an excess of oxalic acid in 1 M-H_2SO_4, was found to be close to 3. The electronic spectrum of Mn incorporated in the vesicles is typical of Mn^{III} (see Figure 2).[9]

On mixing 5 ml of the catalyst solution with 5 ml of 10^{-3} M-$[Ru(bpy)_3]^{3+}$ (pH 3—4) in a glass Rittenberg vessel dioxygen is evolved. The yield of O_2 per oxidant molecule reaches 60—65% (see Figure 3).

Oxidation of Mn^{III} incorporated in the vesicles by an excess of $[Ru(bpy)_3]^{3+}$ leads to the formation of Mn^{IV}; this is shown by the amount of CO_2 formed in the reaction with oxalic acid. Accordingly, a broad asymmetric band typical of Mn^{IV} appears in the electronic spectrum of the catalyst solution in the region of 300 nm (see Figure 2).[9]

Mn^{IV} bound to vesicles is unable by itself to oxidize water to O_2. No O_2 was detected when the catalyst solution was acidified or heated at 80 °C. Dioxygen is evolved only in the presence of the oxidant. Besides $[Ru(bpy)_3]^{3+}$, $[Fe(bpy)_3]^{3+}$ (E 1.05 V)[7] also formed O_2 in the presence of the Mn^{IV} catalyst. No O_2 evolution was observed in the presence of $[Os(bpy)_3]^{3+}$ (E 0.82 V).[7]

Mn^{IV} bound to vesicles may be successfully used as a catalyst for O_2 evolution under the conditions of photochemical generation of $[Ru(bpy)_3]^{3+}$ in the presence of pyrophosphate Mn^{IV} complex as a sacrificial electron acceptor. Irradiation by visible light of a reaction mixture containing 10^{-5} M-$[Ru(bpy)_3]^{3+}$, 10^{-3} M-Mn^{IV} pyrophosphate,[6] and 2×10^{-4} M-Mn^{IV} bound to vesicles in 0.2 M-pyrophosphate buffer (pH 6—8) leads to O_2 evolution with a quantum yield reaching 0.14—0.18. The quantum yield of oxidant generation under these conditions is 0.26.[6]

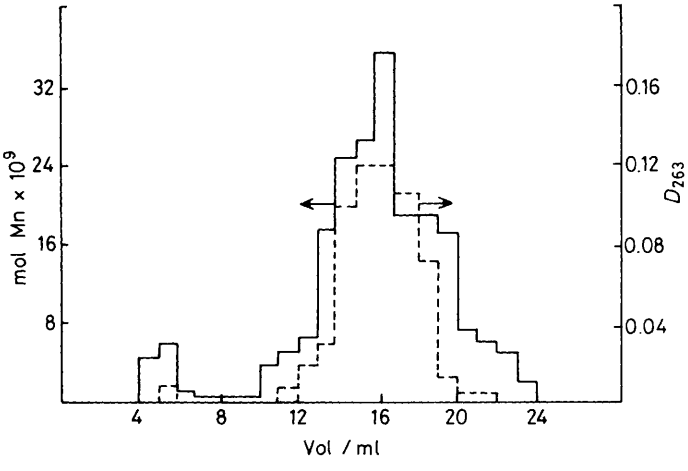


Figure 1. Gel-chromatograms of Mn^{III} bound to vesicles (solid line) and of 'empty' vesicles (dotted line); [DPPC] = 3×10^{-3} M, 0.04 M-borate buffer solution, column 20 × 1 Sepharose 2B.

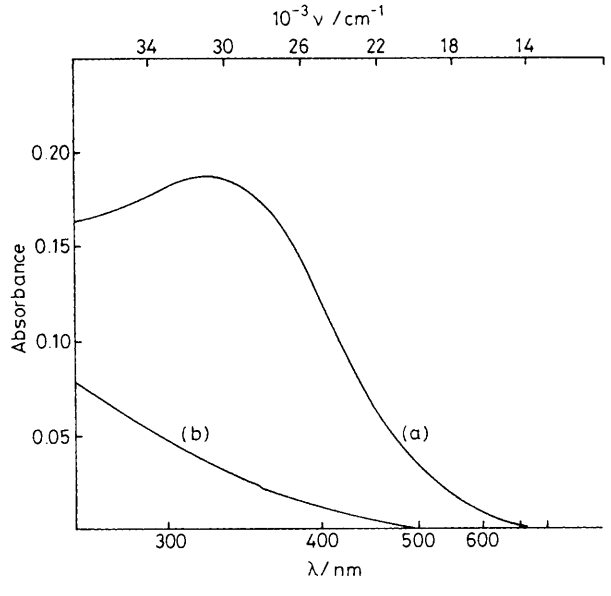


Figure 2. Difference spectra of (a) Mn^{III} and (b) Mn^{IV} bound to vesicles; the comparison cuvette contained Mn^{II} formed by reduction of Mn^{III} and Mn^{IV} by excess of hydrazine; [Mn] = 2.2×10^{-4} M, l = 0.2 cm.

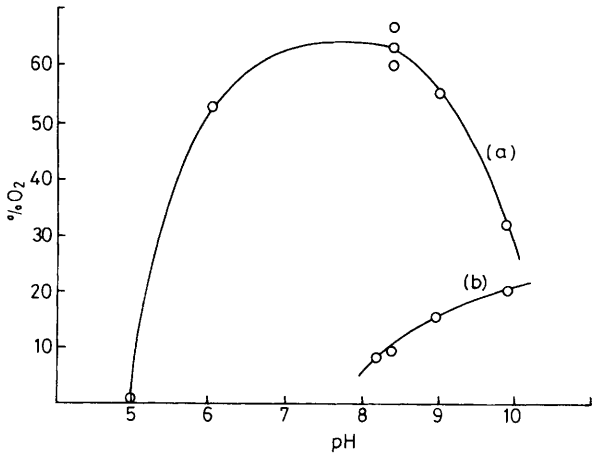
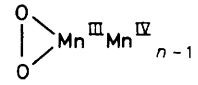


Figure 3. Dependence of O_2 yield on pH in oxidation of water by (a) $[Ru(bpy)_3]^{3+}$ and (b) $[Fe(bpy)_3]^{3+}$ in the presence of Mn catalyst bound to vesicles; $[M(bpy)_3{}^{3+}] = 5 \times 10^{-4}$ M, [borate] = 0.04 M, [Mn] = 1.2×10^{-4} M, V = 10 ml.

Presumably the Mn^{IV} catalyst bound to the vesicles is in the polynuclear state. A one-electron oxidant reacting with the catalyst produces redox changes apparently similar to those found by Dekker[4] for the Mn centre in PSII. Thus in both systems the initial state adapted to O_2 production corresponds to the Mn^{III} oxidation state (S_0 in PSII). The transition $S_0 \rightarrow S_3$ in the PSII Mn centre is accompanied by spectral changes similar to those observed in our system and corresponding to the oxidation $Mn^{III} \rightarrow Mn^{IV}$. The active state of the catalyst for oxidation of water in both natural and model systems may be represented as $A{\cdot}Mn^{IV}{}_n$ or $(A + e)Mn^{V}Mn^{IV}{}_{n-1}$ (A is an electron acceptor) and O_2 formation from two water molecules may involve an intermediate of type (**1**).



(**1**)

The present work demonstrates how the O_2-forming centre may originate in the process of natural evolution. If a lipid membrane is formed in the presence of Mn^{2+} ions they may be easily incorporated and form the catalyst for O_2 evolution from water.

Received, 23rd April 1987; Com. 544

References

1 Govindjee, *Photochem. Photobiol.*, 1985, **42**, 187.
2 K. Sauer, *Acc. Chem. Res.*, 1980, **13**, 249.
3 G. Ch. Dismukes and Y. Siderer, *FEBS Lett.*, 1980, **121**, 78; G. Ch. Dismukes, K. Ferris, and P. Watnick, *Photochem. Photobiol.*, 1982, **3**, 243.
4 J. P. Dekker, H. J. Van Gorkom, M. Brok, and L. Ouwerhand, *Biochim. Biophys. Acta*, 1984, **764**, 301; J. P. Dekker, H. J. Van Gorkom, J. Wensik, and L. Ouwerhand, *ibid.*, 1984, **767**, 1.
5 Oŕ. Hansson and L.-E. Andreasson, *Biochim. Biophys. Acta*, 1982, **679**, 261.
6 V. Ya. Shafirovich, N. K. Khannanov, and A. E. Shilov, *J. Irorg. Biochem.*, 1981, **15**, 113.
7 N. Sutin and C. Creutz, *Pure Appl. Chem.*, 1980, **52**, 2717.
8 V. V. Klimov, S. I. Allakhverdiev, Sh. Demeter, and A. A. Krasnovskii, *Dokl. Akad. Nauk SSSR*, 1979, **249**, 227.
9 C. Lume-Pereira, S. Baral, A. Henglein, and E. Janata, *J. Phys. Chem.*, 1985, **89**, 5772.