Ruthenium Complexes as Electron Acceptors in Photosystem I of Spinach Chloroplasts

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Abbreviations used: DBMIB-2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone; DCMU-3-(3,4-dichlorophenyl)-1, 1-dimethylurea; MV — methylviologen; PS II — photosystem II; PS I — photosystem I; Ru red-ruthenium red; SM — silicomolybdic acid.

Introduction

Ruthenium red is a polymucosaccharide stain, which has been used in mitochondria as a specific inhibitor of Ca^{2+} transport (9, 10). The molecular complex of ruthenium red has been studied by Clausen (7). The crystal structure of the ethylenediamine analog of ruthenium red is shown by Smith *et al.* (13). The reduction of acido ruthenium(III) ammine complexes by various cellular components has been studied by Clarke and associates (5, 6) and as acceptors for NADH dehydrogenases of plasma membrane by Laliberte, Crane and Clarke (8). In this study we have compared the positively-charged ruthenium ammine complexes with the negatively-charged methylviologen as electron acceptors in PS I with the purpose of finding out how local surface charges affect electron transport.

Materials and Methods

Spinach chloroplasts were prepared and assayed as previously described (3). The isolation and suspension medium was 0.4 M sucrose and 0.05 M NaCl (SN chloroplasts). Chlorophyll concentrations were determined according to Arnon (1). Oxygen uptake with methylviologen or oxygen evolution with the ruthenium ammine complexes as electron acceptors was measured with a Clark-type electrode. Reaction rates were recorded with a Sargent-Welch SRG recorder. The ruthenium ammine complexes were synthesized by Dr. Clarke and associates of Boston College with the exception of hexaamine Ru(III) chloride, which was purchased from Alfa Inorganics and further purified by precipitation and recrystalization from 0.1 M HCl. Ruthenium red was purchased from K and K Laboratories, Inc.

Results and Discussion

It was found that chloroplasts vary in their ability to reduce ruthenium ammine complexes and ruthenium red. Figure 1 shows that ruthenium red and chloropentaammine ruthenium(III) gave the lowest electron transport rates in spinach chloroplasts, while the pyridine pentaammine ruthenium complex, which is not as readily autooxidizable, gave the highest rates, comparable to those obtained with methylviologen as the electron acceptor. The differences in rates are also due to reduction potentials of the various Ru(III) complexes and the ability to reach the sites of action in the membrane. The pyridine complex has been shown to penetrate into proteins on account of the pyridine ring (10), thus establishing a closer contact with the active site on the membrane. The optimum pH for all these reactions is between pH 7 and 7.5 (Figure 2).

Since ruthenium compounds have not been used as electron acceptors in

spinach chloroplasts before, it was necessary to establish, if the $H_2O \rightarrow$ MV reaction and the $H_2O \rightarrow$ ruthenium(III) ammine complexes behaved alike toward various PS

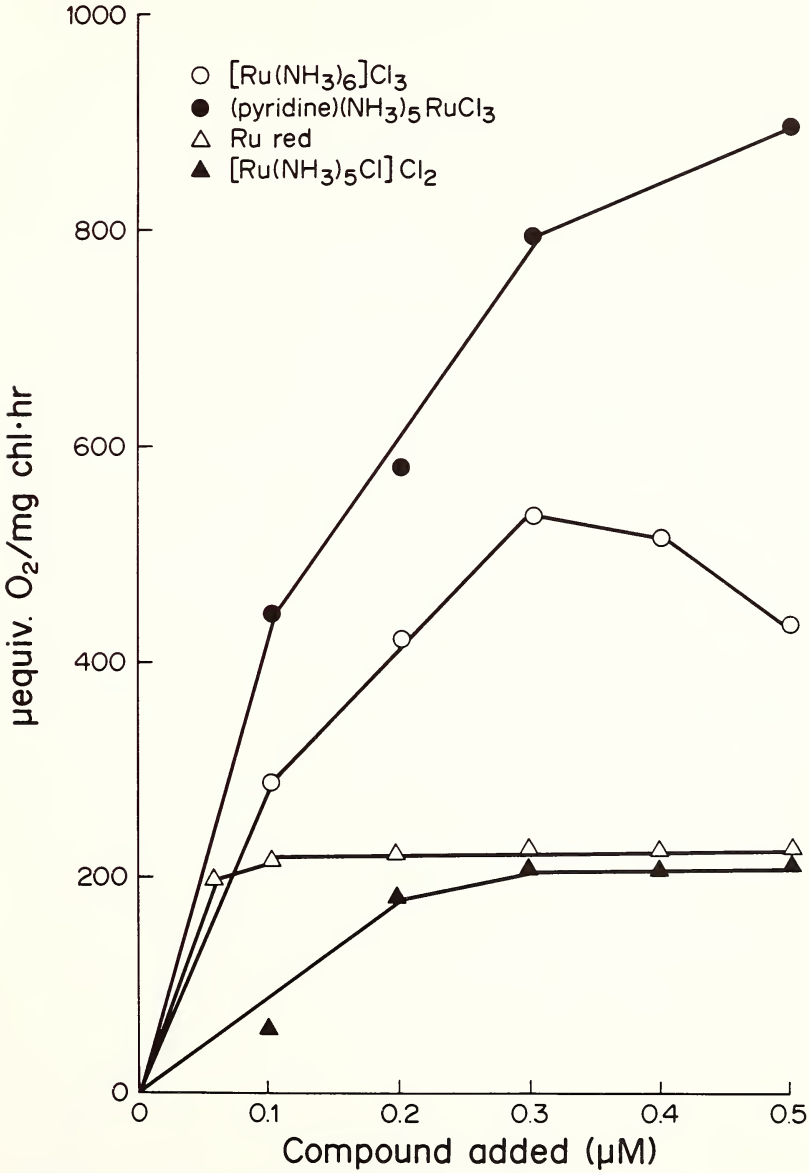

FIGURE 1. *Electron Transport Rates in Spinach Chloroplasts with Ruthenium Ammine Complexes and Ruthenium Red as Electron Acceptors. The reaction mixtures contained chloroplasts (50 μg chlorophyll), 25 mM Tris-Mes buffer, pH 7, 5mM NH_4Cl, 0.5 mM Na azide and ruthenium compounds in concentrations indicated.*

II inhibitors. As Tables I and II show, all reactions tested were inhibited by DCMU, dibromothymoquinone, bathophenanthroline and polylysine, showing that there was no difference between these reactions. This provides evidence that methylviologen and the ruthenium compounds tested act as electron acceptors at the same site or close to each other in PS I.

Since ions can alter local surface charges of membranes, as shown by Barber and associates (2, 12), $MgCl_2$, $CaCl_2$ and bicarbonate ions were tested on the methylviologen and the ruthenium reactions. As Figure 3 shows, Mg^{2+} and Ca^{2+} ions showed very little effect regardless of acceptor used. Bicarbonate, on the other

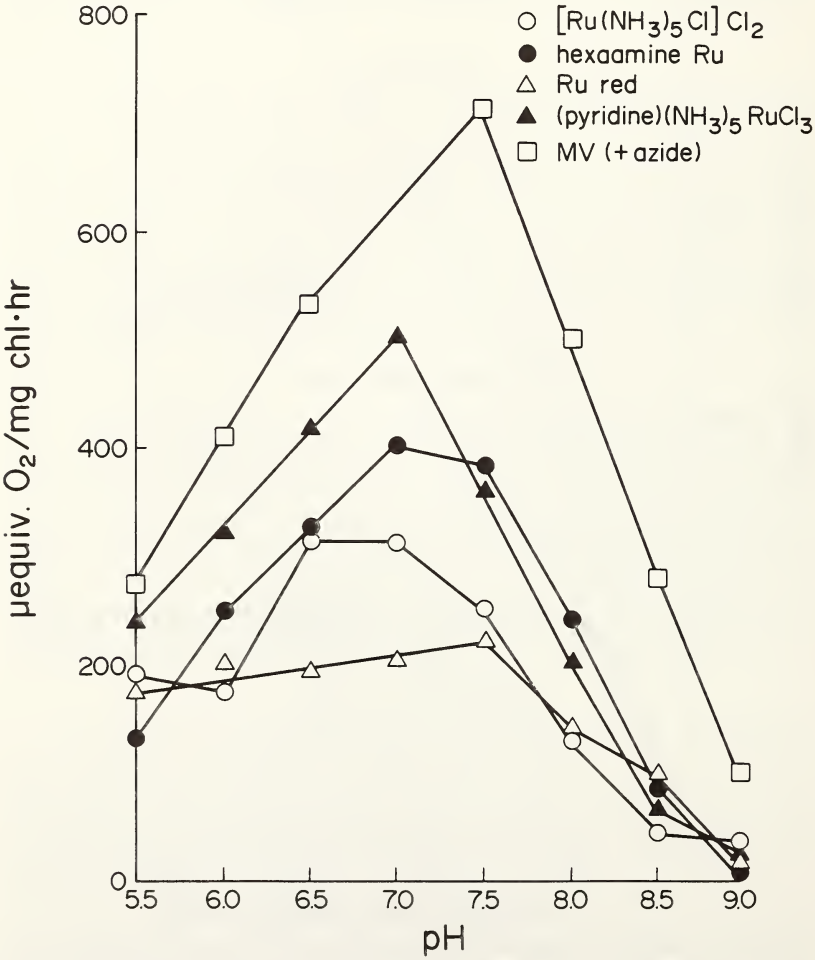

FIGURE 2. *The pH Optima of Electron Transport Reactions with Methylviologen or Ruthenium Compounds as Electron Acceptors. The reaction mixtures were as in Figure 1, except the pH varied as indicated and the optimum concentrations of ruthenium compounds were used.*

TABLE 1. *Inhibition of Ruthenium Electron Transport Pathways in Spinach Chloroplasts*

Inhibitor	Conc.	Electron Transport			
			MV(+ azide)	$[Ru(NH_3)_6]Cl_3$	
		rate[1]	inhibition	rate[1]	inhibition
	(μM)		(%)		(%)
None	—	496	—	463	—
DCMU	5	0	100	51	89
DBMIB	2.5	56	89	113	76
Bathophenanthroline	500	109	78	56	88
Polylysine (35,000 M.W.)	0.2 mg	56	89	113	76

[1] μequiv. O_2/mg chl · hr

hand, showed less than 25% stimulation of rates with ruthenium compounds as electron acceptors in PS I in contrast to 50% stimulation with methylviologen as the acceptor. This may imply that one of the 2 known bicarbonate sites of action in PS II (4, 14) has been eliminated through the alteration of local membrane surface charges. An alternate explanation of the observed facts may be that there is an attraction between positively-charged ruthenium compounds and negatively-charged bicarbonate ions, but this appears unlikely, since the bicarbonate ions were bound to the chloroplast membrane before the addition of the final electron acceptor. Therefore, the inhibition of ruthenium reduction is chloroplasts by bicarbonate appears to arise from an alteration of local surface charges or through some unknown mechanism. Further studies are in progress to define the mechanism of bicarbonate inhibition on the ruthenium but not on the methylviologen pathway in spinach chloroplasts.

In summary, it was found in this study that ruthenium ammine complexes can be used as artificial electron acceptors in PS I of spinach chloroplasts and that the

TABLE 2. *Inhibition of Ruthenium Electron Transport Pathways in Spinach Chloroplasts*

Inhibitor	*Conc.*	*Electron Transport*			
		(Pyridine)	*$(NH_3)_5RuCl_3$*	*Ru red*	
		rate[1]	*inhibition*	*rate[1]*	*inhibition*
	μM		%		%
None	—	964	—	186	—
DCMU	5	62	94	0	100
DBMIB	2.5	141	85	0	100
Bathophenanthroline	500	146	85	65	65
Polylysine (35,000 M.W.)	0.2 mg	146	85	45	76

[1] μequiv. O_2/mg chl · hr

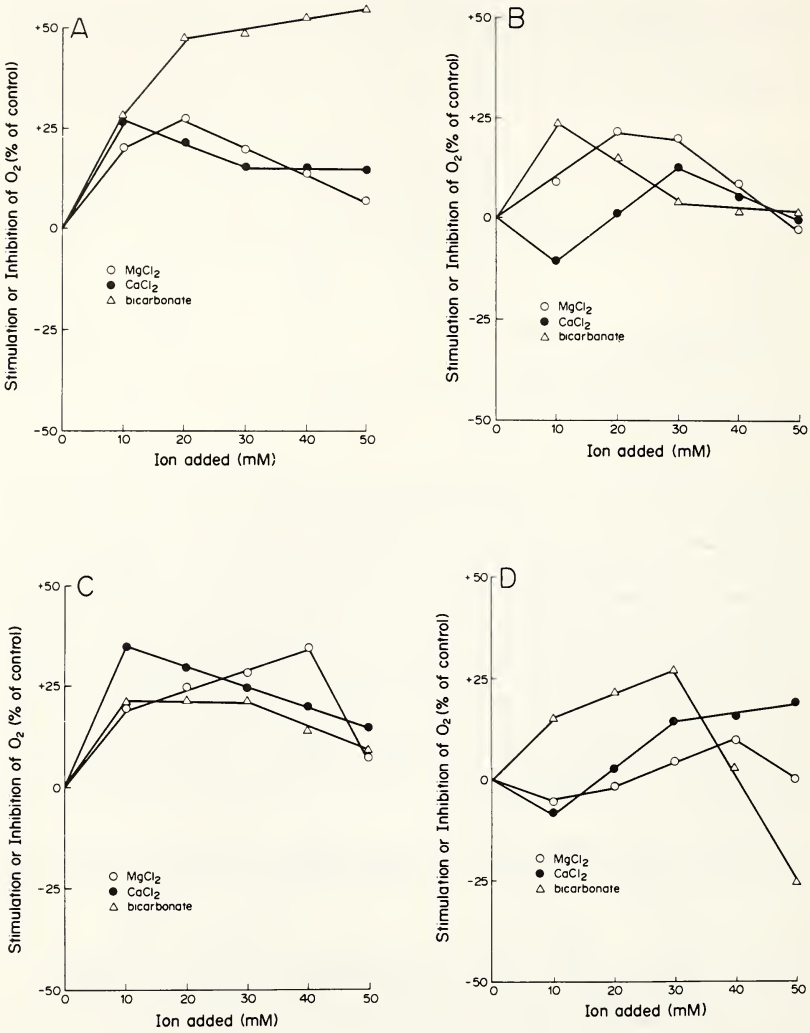

FIGURE 3. *The Effect of Various Ions on the Methylviologen and Ruthenium Compound Electron Transport Pathway in Spinach Chloroplasts. The reaction mixtures contained chloroplasts (50 μg chlorophyll), 25 mM Tris-Mes buffer, pH 7.5 mM NH_4Cl, 0.5 mM Na azide, the optimum concentreation of ruthenium compounds as in Figure 1, and various ions in concentrations indicated.*
A-H_2O → MV(+ azide); B-H_2O → $[Ru(NH_3)_6]\ Cl_3$; C-H_2O → $[Ru(NH_3)_5Cl]\ Cl_2$; D-H_2O → Ru red.

electron transport pathway with methylviologen as the electron acceptor behaves as the ruthenium pathway toward various inhibitors, except with bicarbonate ions.

Acknowledgments

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Literature Cited

1. ARNON, D. I. 1949. Cooper enzyme in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1-15.
2. BARBER, J. and S. MALKIN. 1981. Salt-induced microscopic changes in chlorophyll fluorescence distribution in the thylakoid membrane. Biochim. Biophys. Acta 634, 344-349.
3. BARR, R. and F. L. CRANE. 1981. Phosphate stimulates or inhibits silicomolybdate reduction in spinach chloroplasts. Proceed. Indiana Acad. Sci. 90, 92-97.
4. BARR, R., R. MELHEM, A. L. LEZOTTE and F. L. CRANE. 1980. Stimulation of electron transport from Photosystem II to Photosystem I in Spinach chloroplasts. J. Bioenergetics and Biomembranes 12, 197-203.
5. CLARKE, M. J. 1980. Oncological implications of the chemistry of ruthenium. in *Metal Ions in Biological Systems*, Vol. II, H. Sigel, ed. Marcel Dekker. New York pp. 231-283.
6. CLARKE, M. J., S. BITLER, D. RENNERT, M. BUCHBINDER and A.D. KELMAN. 1980. Reduction and subsequent binding of ruthenium ions catalyzed by subcellular components. J. Inorganic Biochem. 12, 79-87.
7. CLAUSEN, C. A., R. A. PRADOS, and M. L. GOOD. 1971. A M§ossabauer study of ruthenium red. Inorganic Nucl. Chem. Lett. 7, 485-489.
8. LALIBERT\E, J.-F., F. L. CRANE and M. J. CLARKE. 1982. Ruthenium ammine complexes as acceptors for the NADH dehydrogenases of plasma membranes, in preparation.
9. LUTHRA, R., and M. S. OLSON. 1977. The inhibition of calcium uptake and release by rat liver mitochondria by ruthenium red. FEBS Lett. 81, 142-146.
10. MAUK, A. G., R. A. SCOTT and H. B. GRAY. 1980. Distances of electron transfer to and from metalloprotein redox sites in reactions with inorganic complexes. J. Am. Chem. Soc. 102, 4360-4363.
11. MOORE, C. L. 1971. Specific inhibition of mitochondrial Ca^{++} transport by ruthenium red. Biochem. Biophys. Res. Commun. 42, 298-305.
12. RUBIN, B. T., W. S. CHOW and J. BARBER. 1981. Experimental and theoretical considerations of mechanisms controlling cation effects on thylakoid membrane stacking and chlorophyll fluorescence. Biochim. Biophys. Acta 634, 174-190.
13. SMITH, P. M., T. FEALEY, J. E. EARLEY and J. V. SILVERTON. 1971. The crystal and molecular structure of di-μ-oxo-bis (pentaammine ruthenium) bis (ethylenediamine) ruthenium hexachloride. The ethylenediamine analog of "ruthenium red." Inorganic Chem. 10, 1943-1947.
14. STEMLER, A. 1980. Forms of dissolved carbon dioxide required for photosystem II activity in chloroplast membranes. Plant Physiol. 65, 1160-1165.