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PREPARATION AND PROPERTIES OF OSMOGLOBINS. OXIDATION-REDUCTION CATALYTIC ACTIVITY OF RUTHENATED OSMOGLOBIN *

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Reconstitution of native and ruthenium-modified sperm whale myoglobins (Mb and Ru_3Mb) with $[Os^{II}(MIX)(CO)(EtOH)]$ (MIX = mesoporphyrin IX – dicarboxylic acid) and $[Os^{II}(MIX)(DMF)_2]$ yields carbonyl osmoglobin ($[Os^{II}(CO)][Mb]$) and oxidized osmoglobins ($[Os^{III}][Mb]$, $[Os^{III}][Ru_3Mb]$). The visible spectrum of $[Os^{II}(CO)][Mb]$ exhibits α and β bands at 538 and 510 nm, respectively. The ascorbate reduction of dioxygen to water is catalyzed by $[Os^{III}][Ru_3Mb]$.

Derivatives of myoglobin (Mb) made by substituting a metallomacrocycle [2] or another metalloporphyrin [3,4] for the heme or by attaching redox-active inorganic reagents to surface histidines [4–6] have been actively investigated in recent years. It was reported earlier that a pentaammineruthenium(III) derivative of sperm whale myoglobin, Ru_3Mb, is a catalyst for the ascorbate reduction of dioxygen to hydrogen peroxide [6]. We have now extended this study to an osmium derivative, osmoglobin, which is of particular interest because the availability of the higher oxidation states of the 5d metal might lead to novel oxidation-reduction chemistry [7,8].

Carbonyl osmoglobin, $[Os^{II}(CO)][Mb]$, was prepared by adding a slight excess (~ 10%) of $[Os^{II}(MIX)(CO)(EtOH)]$ (MIX = mesoporphyrin-IX-dicarboxylic acid) in a small amount of dimethylformamide (or pyridine) to apoMb (0.1 M) in 0.05 M TrisHCl (pH 7.2) [9]. The resulting solution was left to stand at 0 °C for 2–3 h and then passed through a Sephadex G-25 column that had been equilibrated with 0.01 M trisHCl buffer (the column was eluted with the same buffer). The reconstituted myoglobin was loaded on a CM-52 column and then eluted with 0.1 M trisHCl buffer to give purified carbonyl osmoglobin.

The resonance Raman spectrum of $[Os^{II}(CO)][Mb]$ is similar to that of $[Os^{II}(MIX)(CO)(py)]$ (py = pyridine) in the 1200–1600 cm^{-1} region [10]. The

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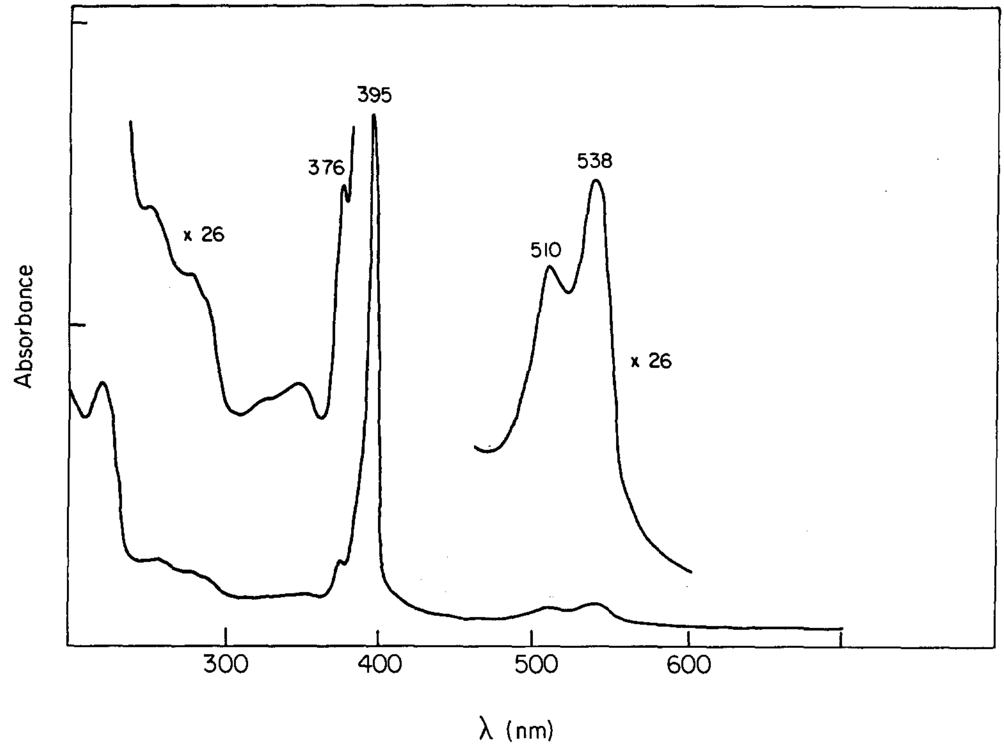

Fig. 1. UV-vis spectra of $[Os^{II}(CO)][Mb]$ (~ 100 mM) in TrisHCl buffer (pH 7.2) at 25 ° C.

electronic absorption spectra of $[Os^{II}(CO)][Mb]$ (fig. 1) and $[Os^{II}(MIX\text{-}DME)(CO)(Im)$ [11] are virtually identical but, unlike $[Ru^{II}(CO)][Mb]$ [3c,d], there is no splitting of the α-band. The finding that identical visible spectra are obtained for $[Os^{II}(CO)][Mb]$ samples prepared from $[Os^{II}(MIX)(CO)(EtOH)]$ and apoMb mixed in different mol-ratios (1 : 1, 3 : 1, 5 : 1) shows that there is one osmium moiety bound per protein molecule. Recent studies have shown that the absorption spectrum of $[Ru^{II}(CO)][Ru_3Mb]$ is similar to that of $[Ru^{II}(MIX\text{-}$

Table 1

Reactivity parameters for the aerobic oxidation of ascorbate catalyzed by $[Os(III)][Ru_3Mb]$ and related species at 25 ° C [pH = 5.5)

Catalyst	K_m (M)	k_{cat} (s^{-1})	Stoichiometry (ascorbate: oxygen)
Ru_3Mb [a]	1.5×10^{-5}	0.60	1:1
$[NH_3)_5RuIm]^{3+}$ [a]	1.3×10^{-5}	0.0035	1:1
$[Os(III)][Mb]$	[b]	[b]	[b]
$[Os(III)][Ru_3Mb]$	3×10^{-5}	0.80	2:1
$[Os^{II}(MIX)(DMF)_2]$	2.6×10^{-5}	0.013	2:1

[a] Reference [6].

[b] Not active.

DME)(CO)(Im)] [12]. Apparently, subtle structural changes associated with surface modification are sufficient to return the heme electronic spectrum to its normal state.

Reconstitution of Mb and Ru_3apoMb [6] with [Os^{II}(MIX)$(DMF)_2$] [9] yields [Os^{III}][Mb] and [Os^{III}][Ru_3Mb], respectively, after aerobic oxidation and purification. The electronic absorption spectra of these oxidized osmoglobins are similar to that of osmium(III)octaethylporphyrin (broad band at ~ 510 nm and a 389 nm Soret) [13]. An interesting finding is that the ruthenated osmoglobin derivative, [Os^{III}][Ru_3Mb], but not [Os^{III}][Mb], catalyzes the 4-electron reduction of dioxygen by ascorbate (table 1), although it is not known whether H_2O_2 is formed in the initial stages of the reaction or not. It is likely that high-valent osmium oxo/peroxo-porphyinato complexes, similar to the oxoiron species in P-450 chemistry [14], are involved in these catalytic reactions.

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[9] [Os^{II}(MIX)(CO)(EtOH)] was prepared by treatment of [Os^{II}(MIX-DME)(CO)(EtOH)] (MIX-DME = mesoporphyrin IX − dimethylester) [8b] with ethanolic KOH. Broad band irradiation of [Os^{II}(MIX)(CO)(EtOH)] in degassed dimethylformamide (DMF) gives [Os^{II}(MIX)(DMF)]$_2$.

[10] Resonance Raman peaks (cm^{-1}): 1228 (m), 1315 (s), 1365 (m, broad) 1390 (m), 1400 (m), 1545 (s).

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