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COLLOIDAL PLATINUM AS AN EFFICIENT AND SELECTIVE CATALYST FOR REDUCTION OF METALLOENZYMES AND METALLOCOENZYMES

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Colloidal platinum supported on a hydrophilic polymer was found a very efficient catalyst for the reduction of a variety of metalloenzymes and metallocoenzyme with molecular hydrogen. Pseudo second order rate constants are ranging from 10^7 to 10^1 $sec^{-1}M^{-1}$ and the selectivity is not simply determined by the corresponding oxidation-reduction potential, suggesting that certain unique interaction exists between the present polymer catalyst and certain metalloenzymes. Methoxy-PMS was found to be a cocatalyst for horseradish peroxidase but not for cyt P-450 which requires a flavoprotein as a specific reductase, again suggesting the presence of specific interaction between the native flavoprotein and P-450.

Metalloenzymes or metallocoenzymes having transition metals in their active sites very often develop their activities only after taking electrons from reductases, hydrogenases or other electron transferring proteins. This very rapid and efficient electron transfer can be achieved *chemically* when very strong reducing agents are used. Recently, we are interested in replacing the very strong reductants by much weaker electron sources such as H_2 in order to avoid any undesirable side reactions. For the purpose, colloidal Pt supported on a hydrophilic polymer may be appropriate as an efficient catalyst, since collidal Pt is well known as an excellent catalyst converting H^+ to H_2[1)-8)], just like a hydrogenase[9)10)]. Our preliminary experiments of using a H_2/colloidal Pt system as a reducing agent were successful, for the reduction of cyt c or cyt c_3[11)] and also for the nonenzymatic reduction of $TPP{\cdot}Mn^{III}$ as a chemical model system of cyt P-450 type O_2 activation[12)].

Now we wish to report that the H_2/colloidal Pt system shows interesting selectivity toward metalloenzymes or -coenzymes, not governed by their oxidation-reduction potentials.

Thus, cyt c_3 from desulfovibrio vulgaris MF (IAM 12604), cyt c from horse heart, met hemoglobin (Hb) from bovin blood, met myoglobin (Mb) from horse heart, cyt P-450 from rabbit liver, catalase from bovine liver, peroxidase from horseradish (HRP) and aquocobalamine ($VB_{12}{\cdot}Co(III){\cdot}H_2O$) were used for the reduction. These enzymes were purified or prepared as described in literatures[13-17)]. A solution of an enzyme or a coenzyme (1.95 ml) was deaerated through careful substitution of air by Ar. A solution of colloidal Pt supported on PVA (final

concentration of Pt is 600 n atom-eqv/mol) freshly prepared[18] was diluted to the desired Pt concentration and H_2 was vigorously bubbled into the resultant solution for 15 min. The H_2 saturated solution thus prepared (0.05 ml) was added into the deaerated enzyme (or coenzyme) solution by the use of a specially designed syringe[19]. The reduction rates of the enzymes or a coenzyme with H_2/colloidal Pt were studied by following the intensity change vs. time at their characteristic absorptions; 416 nm for cyt c, 405 nm for cyt c_3, 406 nm for Hb, 410 nm for Mb, 350 nm for VB_{12}·Co(III)·H_2O, 418 nm for P-450, 406 nm for catalase and 403 nm for HRP.

Among the metalloenzymes or metallocoenzyme used in the experiments, cyt c, cyt c_3, and VB_{12}·Co(III)·H_2O were very easily reduced with H_2/colloidal Pt, while the reduction rates of HRP, P-450 and catalase were very slow. Hemoglobin and myoglobin were between two extremes (see Table 1). Satisfactory reduction of cyt c, cyt c_3, Hb, Mb and VB_{12}·Co(III)·H_2O were

Table 1

enzyme or coenzyme (concentration)	oxidation-reduction potential	conc. of Pt, M	$\tau_{1/2}$
cyt c (10 μM)[a]	+ 0.25[23]	7.5×10^{-7}	6 sec
Hb (3 μM)	+ 0.15[23]	7.5×10^{-7}	15 min
Mb (10 μM)	+ 0.06[23]	7.5×10^{-7}	60 min
VB_{12}·Co(III)·H_2O (66 μM)		7.5×10^{-7}	40 sec
HRP (9 μM)	- 0.27[23]	3.0×10^{-5}	11.9 hr[a]
HRP(9μM) + MeO-PMS(1.8μM)		3.0×10^{-5}	30 min
HRP(9μM) + MeO-PMS(10μM)		3.0×10^{-5}	14 min
cyt c_3 (3 μM)[b]	-0.31 ∿ -0.20[24]	7.5×10^{-7}	15 sec
P-450(5.2 μM)[c]	- 0.33[25]	3.0×10^{-5}	3.9 hr[d]
P-450(2.5μM) + MeO-PMS(10μM)		3.0×10^{-5}	3.9 hr[d]
catalase (6.2 μM)		3.0×10^{-5}	6.7 hr[d]
catalase(6.2μM) + MeO-PMS(10μM)		3.0×10^{-5}	6.7 hr[d]

A catalyst for the reduction; a) cyt-c reductase, b) hydrogenase, c) P-450 reductase, d) These values were estimated by extrapolation from the observed intensity changes after 30 min.

confirmed by the characteristic CD and electronic spectra of the corresponding reduced states. The quantitative recovery of the starting enzymes or coenzyme after reoxidation was similarly confirmed for cyt c, cyt c_3, Mb and VB_{12}·Co(III)·H_2O. Only for Hb, appreciable denaturation of the protein was observed by the CD-ellipticity decrease at 410 nm as well as considerable change in the peak-shape at 222 nm.

As apparent from Table 1, most of the observed reduction rates behave *normally*—i.e. the rate becomes larger as potential becomes more positive (less negative). However, cyt c_3 having

large nagative potential (-0.31 ∿ -0.20 (NHE)) was reduced much faster than Hb (met hemoglobin) or Mb having positive potentials, +0.15 and +0.06 (NHE), respectively. The observed second order rate constant between cyt c_3 and colloidal Pt particle was estimated to be 6.14×10^7 $M^{-1}sec^{-1}$ by assuming that the aggregation number of Pt is 1×10^3 per single colloidal particle[20,21]. This unusually large rate constant suggests the presence of the significant interaction between the present "artificial" hydrogenase (colloidal Pt/H_2O) and cyt c_3, just like the native hydrogenase- cyt c_3 interaction.

For the slowly reducible metalloenzyme a flavin analog, MeO-PMS (+0.063, NHE)[22], was used as a likely cocatalyst, by considering that flavins often behave as active sites of heme-protein reductases. Interestingly, cocatalytic effect of the flavin analog was again very selective---i.e.---MeO-PMS was very active toward HRP even in a catalytic amount but practically inactive toward P-450 and catalase. Although P-450 requires a flavoprotein as the specific reductase, MeO-PMS does not accelerate the reduction (activation), strongly suggesting that certain specific protein-protein interaction is important for the P-450 reduction. In order to confirm that colloidal Pt is still active in the solution after the catalytic reduction, 0.1 ml of 4 mM solution of methylene blue was added to 2 ml of solution containing colloidal Pt and HRP, P-450 or catalase. Methylene blue (final concentration was 190 μM) was reduced immediately after the addition, demonstrating that colloidal Pt keeps its activity perfectly after the reduction. CD spectra of the oxidized form (Fe^{III}) of catalase or P-450 was not affected appreciably in the presence of colloidal Pt before as well as after the reduction, showing that neither serious conformation change nor serious electronic state perturbation took place in the enzymes in the presence of colloidal Pt.

From these results, interesting catalytic activity of colloidal Pt toward enzyme or coenzyme reduction with H_2 was firstly observed. The activity depends on the nature of the metalloenzymes or the metallocoenzyme and thus the catalytic reduction is very selective.

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