

ganded with pyridine or nicotinic acid, the reduction does not occur. Either heme or heme-proteins transferred into sterile cuvettes through 5 μ Millipore filters become reduced at the same rate as unsterile solutions. It is notable that cytochrome c oxidase, with multiple electron accepting sites, shows a much faster rate of reduction than other proteins studied.

EXAMPLE 6.

CO-Driven Hemoglobin Reduction

The half-time of reduction of metHbA under CO is dependent on CO concentration. The time necessary for half-reduction is decreased 25-fold by an increase in CO pressure from one to 100 atmospheres. Table II shows that the rate of reduction is different for hemoglobin and myoglobin. To see if the alpha and beta chains of HbA also differed in their rate of reduction, we examined the spectral dependence of the CO recombination after flash photolysis at varying stages of the reductive process. Because the kinetics of recombination differ for the alpha and beta subunits, the contribution to the total absorbance change from each subunit can be determined at different wavelengths. At 437.5 nm, both chains contribute about equally to the total absorbance change. FIG. 2 shows a representative time course for the absorbance change at 437.5 nm, upon CO recombination after complete flash photolysis of partially reduced HbA. Since the fast and slow fractions observed for partially reduced samples of Hb are of approximately equal magnitude at this wavelength, we conclude that the alpha and beta subunits are reduced at similar rates.

Polyanions such as inositol hexaphosphate (IHP) shift the conformational equilibrium of HbA towards its low affinity (T) conformation. Under one atmosphere of CO, a small molar excess of IHP, about 1%, over heme consistently increases the rate of HbA reduction; decreasing the half-time for reduction by about 20%. Under 100 atmospheres of CO, the effect is noticeable only after about 75% of the HbA is reduced, at which point the rate of HbA reduction without IHP declines sharply. With IHP present, the rate of reduction decreases less quickly after the first three out of four heme groups are reduced.

EXAMPLE 7

CO-Driven Hemin Reduction

As documented in Table II, hemin, freshly dissolved in basic solution, is reduced and binds CO when incubated under an atmosphere of CO. FIG. 3 shows the time courses of the reaction at varying concentrations of KOH. The rates for hemin reduction, as for metHbA reduction, vary during the course of reduction, giving distinctly sigmoidal curves when the percent reduction is plotted as a function of time. For hemin, the rates are symmetrical around the half-time, and average rates were therefore equal to the reciprocal half-times. As further shown in FIG. 3, for a given concentration of hemin, the rate of reduction is base-concentration dependent. Oxidized hemin dissolved in air equilibrated KOH solution for several hours prior to degassing and saturation with CO does not become reduced as quickly as freshly dissolved hemin. After "aging" for 24 hours, the hemin solutions do not become reduced under CO at any measurable rate.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within

the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

Well known oxidants can be found in the following references which are hereby incorporated in this application:

- (1) Handbook of Biochemistry, Chemical Rubber Co., 2nd Edition, 1973;
- (2) Handbook of Chemistry & Physics, Chemical Rubber Co., 52nd Edition, 1971.

What is claimed as new and desired to be secured by Letters Patent of the United States is:

1. A method of oxidizing NO to NO₂, comprising:

- (i) contacting, together, NO, a nitrogen-containing chelating agent and water; wherein said chelating agent is at least one member selected from the group consisting of porphines, hemes, phthalocyanines, ethioporphyrins, sirohemes, and derivatives thereof, bound to a support, wherein said support is glass, a natural fiber, a synthetic fiber, a gel, charcoal, carbon ceramic material, a metal oxide, a synthetic polymer, a zeolite, a silica compound or an alumina compound; and

(ii) obtaining NO₂.

2. The method of claim 1, wherein said NO is in the gas phase.

3. The process of claim 1, wherein said nitrogen-containing chelating agent is methemoglobin.

4. The method of claim 1, wherein said nitrogen-containing chelating agent is ferric hemoglobin.

5. The method of claim 1, wherein said nitrogen-containing chelating agent is sperm whale myoglobin.

6. The method of claim 1, wherein said support is glass, a natural fiber, a synthetic fiber or a gel.

7. The method of claim 1, wherein said support is charcoal, a ceramic material or a metal.

8. The method of claim 1, wherein said support is a metal oxide, a synthetic polymer, zeolite, a silica compound or an alumina compound.

9. The method of claim 1, comprising oxidizing NO to NO₂ in the presence of methylene chloride, DMF, DMSO, pyridine, methylimidazole, benzonitrile, dichloroethane, THF, propylene carbonate, chloroform, carbon tetrachloride, benzene, toluene, acetonitrile, a C₁₋₃ alcohol, acetone, a C₁₋₄ amine, a C₂₋₆ thiol, a C₂₋₆ mercaptan, ammonia, or a C₂₋₆ ether.

10. The method of claim 1, comprising using a pH of from 4 to 14.

11. The method of claim 1, comprising using a pH of from 6 to 10, and a buffer.

12. The method of claim 1, wherein said nitrogen-containing chelating agent is an iron-containing porphyrin.

13. A method for oxidizing NO to NO₂, comprising:

- (i) contacting, together, NO, a nitrogen-containing chelating agent and water; wherein said chelating agent is heme bound to a support, or wherein said chelating agent is hemin, and said support is glass, a natural fiber, a synthetic fiber, a gel, charcoal, carbon, a ceramic material, a metal, a metal oxide, a synthetic polymer, zeolite, silica compound or an alumina compound; and

(ii) obtaining NO₂.

14. The method of claim 13, wherein said nitrogen-containing chelating agent is heme.

15. The method of claim 13, wherein said nitrogen-containing chelating agent is hemin.