Transition Metal Complexes of 1,10-Phenanthroline-5,6-dione as Efficient Mediators for the Regeneration of NAD$^+$ in Enzymatic Synthesis

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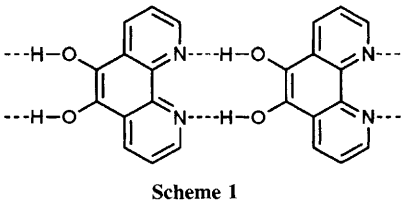
The complexation of 1,10-phenanthroline-5,6-dione by transition metals increases the catalytic turnover frequency by a factor of up to 17 for the aerobic-chemical and a factor of 14 for the indirect electrochemical NAD$^+$-regeneration for HLADH catalysed alcohol oxidations in comparison with the uncomplexed phenanthrolinediones.

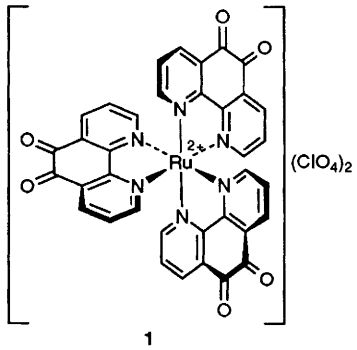
For synthetic application of NAD(P)$^+$-dependent oxidoreductases in oxidation reactions an efficient system for the cofactor regeneration is necessary.[1] Besides using enzymatic cofactor recycling systems, chemical and electrochemical regeneration procedures have attracted strong interest, because of their greater flexibility.[1,2] Chemical regeneration systems usually consist of quinoid structures and oxygen acts as final electron acceptor in combination with catalase to destroy the hydrogen peroxide produced. Electrochemical regeneration of the oxidised cofactor is possible either by direct anodic oxidation[3] or *via* redox catalysis, mainly applying *o*-quinoid systems as mediators.[4] However, all these systems have limited use because they react too slowly (FMN:[5] 1.8 turnovers h^{-1}; 4,7-phenanthroline-5,6-dione:[6,7] 4–6 turnovers h^{-1}), the anodic potential for the electrochemical regeneration is in many cases too positive for selective oxidations[3,8] or the systems are not sufficiently chemically stable under the basic conditions,[9] which usually are favourable for these enzymatic oxidations.

To solve these problems, it was our strategy to accelerate the hydride transfer to *o*-quinoid structures by complexation with a transition metal ion, thus lowering the electron density within the ligand. Charged metal complexes also enhance the solubility of the mediators. As the chelating ligand we used 1,10-phenanthroline-5,6-dione. The Co^{II}, Ni^{II} and Cu^{II} complexes reacted under fast hydride ion transfer from NADH to the ligand, forming an insoluble precipitate. This was similar to the behaviour of the uncomplexed ligand. Thus, the application of these systems as mediators for the NAD$^+$-regeneration is excluded. In the case of the free ligand, the precipitation can be explained by oligomerisation or telomerisation of the reduced form by hydrogen bridges between the hydroquinone structure and the imine nitrogens (Scheme 1). The oligomerisation in the case of the metal complexes is still under investigation.

We are proposing two efficient systems which are stable and do not undergo telomerisation. In the first case, the tris(1,10-phenanthroline-5,6-dione) ruthenium(II) perchlorate **1** is used



Scheme 1



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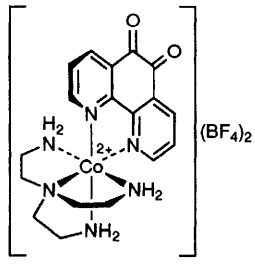

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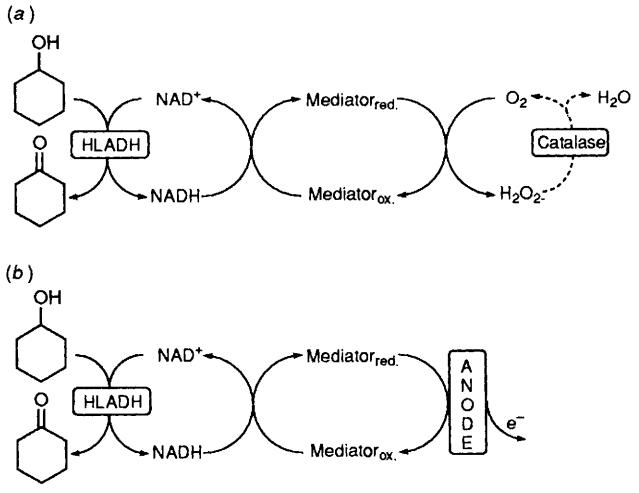

Scheme 2

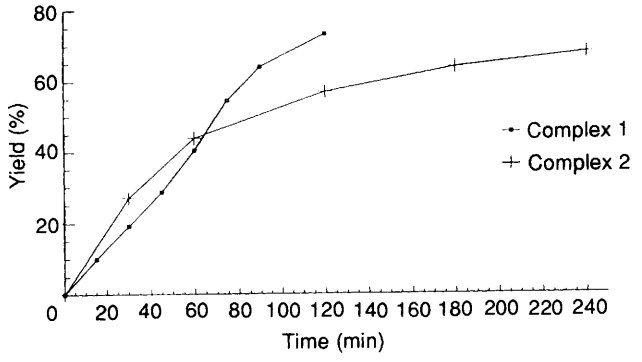

Fig. 1 HLADH-catalysed conversion of cyclohexanol to cyclohexanone under aerobic regeneration of NAD^+ mediated by complex **1** and **2**: 0.1 mol dm^{-3} phosphate buffer of pH 8.2 under oxygen atmosphere at room temperature containing mediators **1** and **2** (1×10^{-4}), NAD^+ (5×10^{-4} mol dm^{-3}), cyclohexanol (2×10^{-2} mol dm^{-3} for **1** and 8×10^{-3} mol dm^{-3} for **2**), HLADH [50 U (EtOH) for **1** and 25 U for **2**] and catalase (3×10^5 U for **1** and 1.3×10^6 U for **2**).

as stable redox catalyst. In the other case, we prevented the telomerisation of the reduced Co^{2+} complex by using a mixed ligand system consisting of 1,10-phenanthroline-5,6-dione (phendi) as the catalytically active unit and one *N,N,N*-tris(aminoethyl)amine (tren) ligand to block the other sites of the metal centre: $[Co(tren)(phendi)](BF_4)_2$ **2**.

Under aerobic conditions using a phosphate buffer of pH 6.8 and 8.2, complexes **1** and **2** produced NAD^+ from NADH at rates of 7.2 (complex **1**, pH 6.8), 35 (complex **1**, pH 8.2), 13.5 (complex **2**, pH 6.8) and 117 turnovers h^{-1} (complex **2**, pH 8.2) after 60 min. Also, electroanalytical experiments using cyclic voltammetry showed strong catalytic oxidation peak enhancements in the presence of NADH when complex **1** ($E_{p,ox} = -0.05$ V *vs.* Ag/AgCl) or complex **2** ($E_{p,ox} = +0.02$ V *vs.* Ag/AgCl) were used in phosphate buffer of pH 8.2 for **1** and pH 6.7 for **2**. We then coupled the regeneration systems to the horseliver alcohol dehydrogenase (HLADH) catalysed oxidation of cyclohexanol to cyclohexanone as a model. Under aerobic conditions using complexes **1** and **2**, catalase was added to destroy the hydrogen peroxide according to Scheme 2*a*. In the case of the anaerobic regeneration using the anode as electron acceptor according to Scheme 2*b*, only complex **1** was tested. The time-dependent formation of cyclohexanone for the aerobic and for the anaerobic electrochemical system is shown in Figs. 1 and 2.

The turnover frequencies for the enzymatic production of cyclohexanone under aerobic conditions after 60 min gave

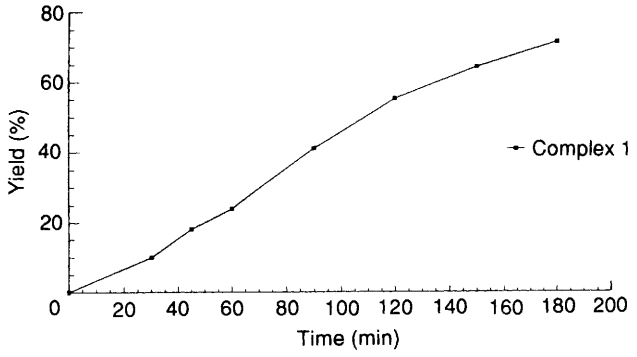

Fig. 2 HLADH-catalysed conversion of cyclohexanol to cyclohexanone by anaerobic electrochemical regeneration of NAD^+ mediated by complex **1**: 0.1 mol dm^{-3} phosphate buffer of pH 8.0 at room temperature under argon atmosphere containing **1** (1×10^{-4} mol dm^{-3}), NAD^+ (5×10^{-4} mol dm^{-3}), cyclohexanol (1×10^{-2} mol dm^{-3}) and HLADH [25 U (EtOH)]. The working potential was 100 mV *vs.* Ag/AgCl with a Sigraflex carbon foil anode of 23 cm^2 surface area and a cell volume of 20 ml.

values of 35 turnovers h^{-1} (complex **1**) and 81 turnovers h^{-1} (complex **2**). These values are larger by a factor of 7 to 17 than those reported for systems using 1,10-phenanthroline-5,6-dione in the presence of diaphorase and catalase.[6] In the case of the anaerobic electrochemical regeneration using complex **1**, we attained 28 turnovers h^{-1}. This value is larger by a factor of 14 than that reported for the indirect electrochemical oxidation using 1,7-phenanthroline-5,6-dione as mediator.[10] The oxidation potentials of the metal complexes are similar to those of the uncomplexed phenanthrolinediones, thus fulfilling the prerequisite for the desired selective oxidation.

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