Reduction of ferri-protoporphyrin IX dimethyl ester by the 1, 2-dihydropyridine type redox resin

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A redox-resin containing 1,2-dihydropyridine residues was synthesized by the reduction of crosslinked poly(*N*-ethylpyridinium bromide) with sodium borohydride. The redox resin was used to reduce the central ferric-iron of ferri-protoporphyrin IX dimethyl ester in heterogeneous solutions.

INTRODUCTION

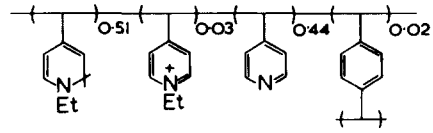
It is well known that ferro-protoporphyrin IX plays an important part as an oxygen carrier in biological systems[1]. The ferro-derivative prepared by the reduction of the ferri complex with inorganic reductants such as sodium hydrosulphite in homogeneous solution is slightly soluble in common organic solvents.

We have shown[2,3] that the reduction of ferri-protoporphyrin IX by polymeric 1,4-dihydronicotinamide derivatives[4,5] in homogeneous organic solvents is more effective than reduction by the monomer. As a development of our work, this paper describes attempts to reduce ferri-protoporphyrin IX dimethyl ester by the use of a crosslinked poly(*N*-ethyl-1,2-dihydropyridine) resin in heterogeneous solution in order to prepare an uncontaminated solution of the ferro complex. The 1,2-dihydropyridine resin was synthesized by the reduction of partly quarternized poly(4-vinylpyridine) beads.

EXPERIMENTAL

Synthesis of crosslinked poly(N-ethyl-1,2-dihydropyridine) redox resin

The Sumichelate CR-2 type poly(4-vinylpyridine) resin crosslinked by 2% divinylbenzene in the form of white bead (50–150 mesh) from Sumitomo Kagaku Co. Ltd (Japan) was immersed and washed with methanol and dried *in vacuo* at 70°C before use. The dried resin (10 g) was immersed in freshly distilled dioxane (100 ml) for a day and then refluxed with ethyl bromide (20 ml) by stirring. The beads were washed with hot dioxane and dried *in vacuo* at 70°C for a day. The content of the quarternized pyridinium residue in the resin determined by the Volhard method was 54 unit mol %. The method for preparing *N*-phenyl-1,2-dihydropyridine[6] was used to the synthesis of a redox-resin. The partly quarternized resin (1.0 g) was added to a stirred solution (200 ml) containing potassium hydroxide (0.5 g) and sodium borohydride (0.88 g), stirring continued at room temperature for 24 h under argon. The resin changed in colour to reddish-brown and then gradually to pale yellow. The reduced resin was washed with nitrogen gas-presaturated water until the elute was neutral and then dried *in vacuo* at 70°C for 40 h. The redox-resin contained 51 unit mol % of *N*-ethyl-1,2-dihydropyridine residue and 3 unit mol % of *N*-ethylpyridinium bromide residue as determined by the Volhard method.



The complete removal of sodium borohydride from the redox-resin, was demonstrated by immersing the resin in water under nitrogen for three days. The supernatant solution showed no precipitation by adding a silver nitrate aqueous solution.

Materials

Commercial-grade dimethyl sulphoxide was distilled under reduced pressure after treating with molecular sieves 4A. Toluene was refluxed over metallic sodium for three hours and distilled. *N*-Ethylimidazole was purified by distillation *in vacuo* after the dehydration with potassium hydroxide pellets and redistilled immediately before use. Ferri-protoporphyrin IX dimethyl ester was prepared according to the method of Adler *et al.*[7] and purified by recrystallization from a dichloroethane/n-hexane mixture. Calculated for $C_{36}H_{36}N_2Cl_1Fe_1$: C = 63.58%; H = 5.34%; N = 8.24%; Cl = 5.19%. Found: C = 63.8%, H = 5.40%; N = 8.20%; Cl = 5.16%. Reagent-grade 2,6-dichlorophenolindophenol sodium was used without purification.

Apparatus

Visible absorption spectra were measured by a Union Giken SM-401 Spectrophotometer with a temperature controlled attachment. The redox reaction was carried out using a vacuum cell containing two vessels separated by glass filter.

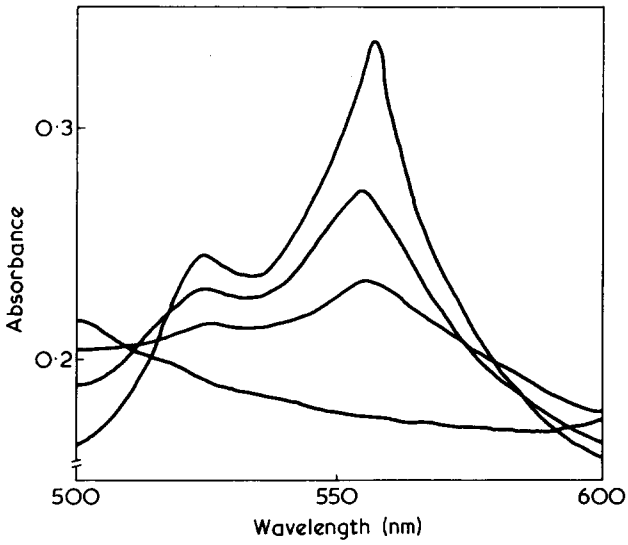
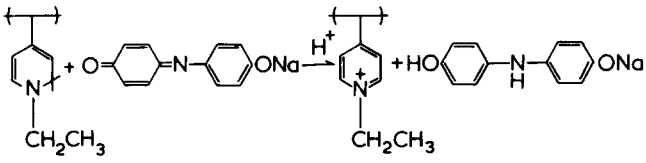


Figure 1 Change of visible absorption spectra in the reduction of ferri-protoporphyrin IX dimethyl ester by the 1,2-dihydropyridine type redox-resin in dimethyl sulphoxide at 25°C. [Ferri-porphyrin]$_0$ = 2.9 X 10^{-5} mol/l; [dihydropyridine residue (effective)]$_0$/[ferri-porphyrin]$_0$/ = 10. Time (h): 0, 1.5, 7.5, 16.0

RESULTS AND DISCUSSION

Determination of the content of redox-residue available to the reduction of large substrates

In the case of a crosslinked resin, polymer chains are constricted by crosslinkings, so that large substrates are not expected to react with the internal residues of a resin. The content of the redox-residue available to the reduction was determined by the use of 2,6-dichlorophenolindophenol sodium since it was reported that this dye is quantitatively decoloured by the reaction by dihydropyridine derivatives[8].



The reduction by the resin was carried out in a 0.007 M phosphate buffer/methanol (pH 7.0) mixture at room temperature under argon. By measuring the absorbance at 529 nm, it was calculated that 7.7 x 10^{-5} equiv. mol of the reductant residue per 1 g of the resin was available to the reduction, while the total reductant residue of the resin was 4.1 x 10^{-3} equiv. mol/g. Therefore, 1/53 of reductant residue is available practically. Since ferri-protoporphyrin IX dimethyl ester is larger in size than 2,6-dichlorophenolindophenol sodium, it is expected that the amount available for the reduction of the porphyrin complex is smaller than that for 2,6-dichlorophenolindophenol sodium.

Reduction of ferriprotoporphyrin IX dimethyl ester by the redox-resin in dimethyl sulphoxide

After evacuation by a freeze–thaw method in a vacuum cell, the dimethyl sulphoxide solution of ferriprotoporphyrin IX dimethyl ester was mixed with the redox beads and its visible absorption spectrum was measured. As shown in *Figure 1*, the absorption due to the ferri-porphyrin(dimethyl sulphoxide)$_2$ complex decreased without isosbestic points while that due to the ferro-porphyrin (dimethyl sulphoxide)$_2$, of which absorption maxima appeared at 526 and 557 nm, increased with time. Therefore, it was confirmed that ferri-protoporphyrin IX dimethyl ester can be reduced to the ferro complex by the 1,2-dihydropyridine resin. The absorbance attained a maximum corresponding to 60% reduction of the total porphyrin. This can be explained by the adsorption of the ferric and ferro-complex to the resin by coordination or electrostatic bond since there was no isosbestic points in the spectrum and also the resin changed in colour from pale-yellow to red-brown.

Reduction of ferric-protoporphyrin IX dimethyl ester in toluene in the presence of N*-ethylimidazole*

The reduction was then carried out in toluene in the presence of excess *N*-ethylimidazole as axial ligand. As shown in *Figure 2*, the reduction to the ferro-porphyrin (*N*-ethylimidazole)$_2$ complex was confirmed by the appearance of the characteristic absorption bands (λ_{max} = 530 and 560 nm). No adsorption of the porphyrin complexes to the resin was observed because the isosbestic points appeared at 508 and 572 nm. This difference between dimethyl sulphoxide and toluene containing *N*-ethylimidazole can be explained by considering competitive equilibria of the complex formation of the ferro- and ferri-porphyrins with the resin and axial ligand in solution. In the latter solution, the complex formation was favoured in solutions containing excess strong ligand, while in the former solution complexing was with the beads rather than with weak ligand dimethyl sulphoxide. The change of absorbance with time and the pseudo first-order plot is shown in *Figure 3*. It has been reported that ferri-protoporphyrin IX is reduced in pure pyridine very slowly[9]. However, in the present solution, the rate constant of the spontaneous reduction by ligand in the absence of the reductant resin was 10^{-3} min^{-1}. Since this value was much smaller than that of the reduction by the redox-resin (k_{obs} = 2.7 x 10^{-1} min^{-1} at 20°C) no difficulties arose for the determination of the rate of reduction by the resin.

Application of the column method to the reduction

A toluene solution of the ferri-complex containing 0.1 mol/l *N*-ethylimidazole was added slowly under nitrogen

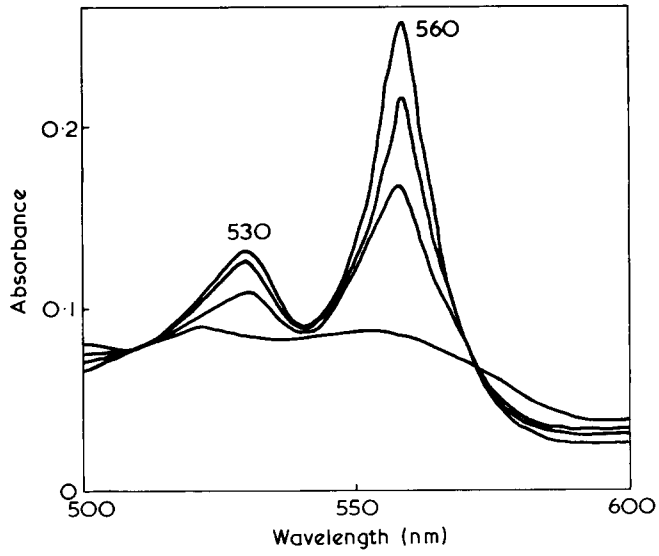


Figure 2 Change of the visible absorption spectra in the reduction of ferri-protoporphyrin IX dimethyl ester by the 1,2-dihydropyridine type redox-resin in toluene at 25°C. [Ferri-porphyrin]$_0$ = 1.5 X 10^{-5} mol/1; [*N*-ethylimidazole]$_0$ = 0.1 mol/1; [dihydropyridine residue (effective)]$_0$/[ferri-porphyrin]$_0$ = 100. Time (min): 0, 1, 5, 10

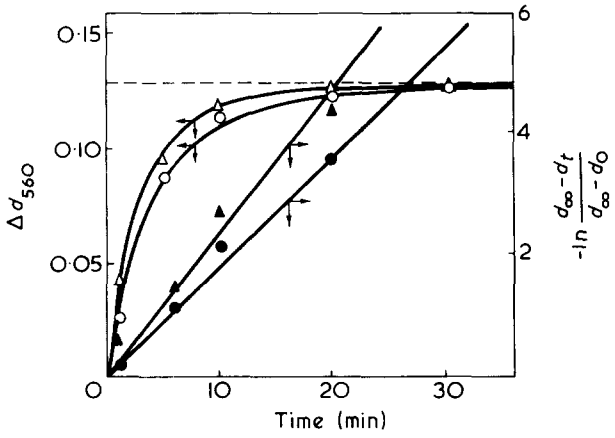


Figure 3 Absorbance changes at 560 nm with time in the reduction of ferri-protoporphyrin IX dimethyl ester in toluene and pseudo first order plots. The experimental conditions were the same as in *Figure 2*. – – – –, theoretical value of Δd for complete reduction. Temperature (°C): 25.0 (△,▲); 20.0 (○,●)

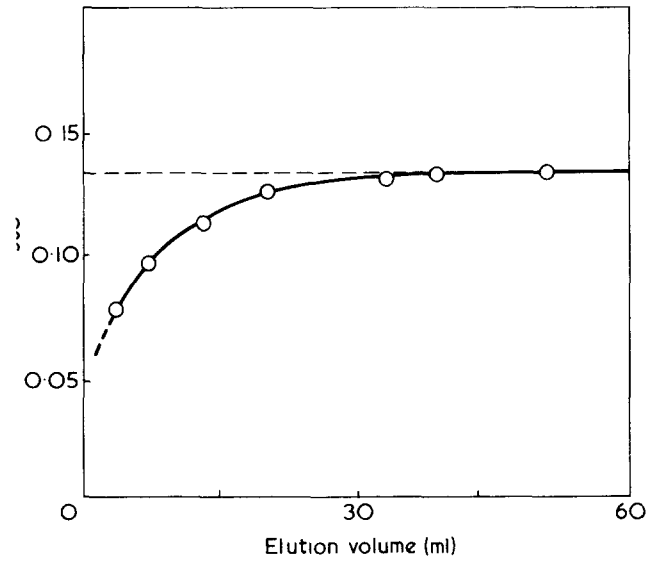


Figure 4 Reduction of ferri-protoporphyrin IX dimethyl ester by the column (0.6 × 20 cm) containing the redox-resin (2.0 g). The toluene solution containing the ferri-porphyrin (1.5×10^{-5} mol/l) and *N*-ethylimidazole (0.1 mol/l) was added from the top of the column (0.6 ml/min)

atmosphere to the top of a column (0.6 x 20 cm) packed with resin-beads: the elute showed the characteristic spectrum due to the ferro-complex and attained constant absorbance, indicating the complete reduction (*Figure 4*).

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