Electrical 'Wiring' of Glutathione Reductase: an Efficient Method for the Reduction of Glutathione using Molecular Hydrogen as the Reductant

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The enzyme glutathione reductase is chemically modified to become electrically conductive, thus facilitating the reduction of oxidized glutathione to its reduced from using hydrogen as the reductant, and the modified enzyme and Pt colloid as catalysts.

In most redox enzymes the active site is shielded by an insulating protein shell, which prevents the transfer of electrons from an external source. It is thus necessary to establish electrical communication between the active site of the enzyme and its environment, by chemically attaching electron relays to the enzyme. It is widely accepted that electrons can 'hop' from one relay to another across the protein structure.[1] Hence, only few electron relay components have to be attached to the protein in order to facilitate electrical communication. The chemical modification that is necessary is therefore minor and does not alter the protein structure or its catalytic activity. Heller[2,3] and coworkers showed that such modification of glucose oxidase leads to an effective electron transfer between the enzyme and an electrode.

Electrical 'wiring' of redox enzymes[2] has been investigated in recent years predominantly as an essential step in the development of biosensors. Consequently, chemical modification of redox enzymes has been focused towards electrical communication between an electrode and a redox enzyme. However, modification of redox enzymes by relay components can greatly contribute to developing new biocatalysed transformations. Here we report on the electrical wiring of the enzyme glutathione reductase, which facilitates the reduction of glutathione with hydrogen as the reducing agent. In nature, glutathione reductase utilizes the NADPH cofactor (NADPH = nicotinamide adenine dinucleotide phosphate) as the electron carrier for its biocatalytic performance. We find that with the electrically modified enzyme the native cofactor can be excluded and the enzyme still maintains its function.

The enzyme glutathione reductase (E.C. 1.6.4.2, obtained from Sigma, type III from bakers yeast, 175 units mg^{-1} protein) is modified by 4,4′-bipyridinium-1,1′-dipropionate, PAV (synthesized according to published procedure[4]), using 1-methyl-3-[3-(dimethylamino)propyl]carbodiimide, EDC, as the coupling agent.[5] PAV (4.0 mg), HEPES {160 mg; sodium salt: [*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulphonic acid)]} and urea (360 mg) are dissolved in water (3.2 ml). The solution is brought to pH 7.0 by the addition of HCl 1 mol dm^{-3}. This aliquot (1 ml) is mixed with 0.05 mol dm^{-3} HEPES buffer solution (1 ml, pH 7.1) containing the enzyme (11.2 mg), and the reaction is initiated by the addition of EDC

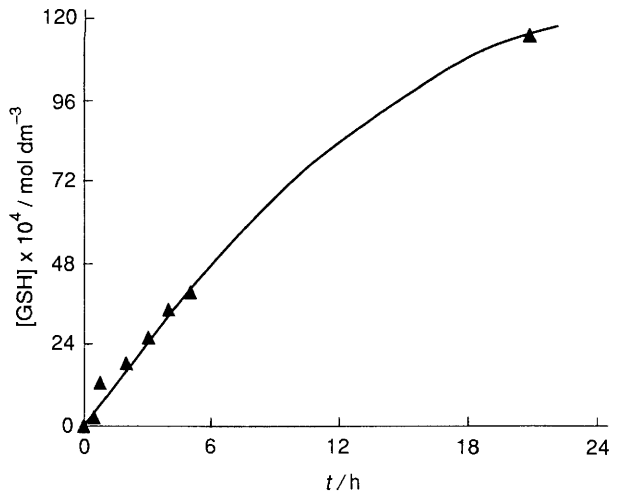

Fig. 1 Rate of GSH evolution in the system, under 1 atm. of H_2. The system contains 3.1 mg of bipyridinium-modified protein, 1.2×10^{-2} mol dm^{-3} GSSG and Pt colloid (70.6 mg l^{-1} Pt).

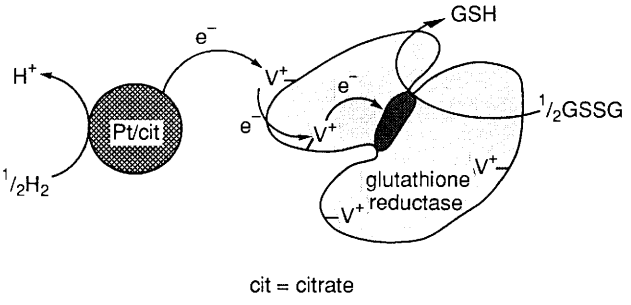

Fig. 2 Schematic description of the catalytic cycle for the reduction of GSSG to GSH by H_2

(13.3 mg). The reaction is stirred at 0 °C for 3 h and the solution is extensively dialysed against 0.1 mol dm^{-3} NaH_2PO_4 buffer, pH 7.5, until no free bipyridinium salt is present in the modified enzyme solution. The resulting solution contains 6.1 mg ml^{-1} of protein,† and the concentration of the protein-bound bipyridinium salt in the solution is estimated to be 1.8×10^{-4} mol dm^{-3}, corresponding to 3.5 molecules of PAV per enzyme molecule‡ (based on molecular weight of 118000 for the enzyme[6]).

The system for the reduction of oxidized glutathione, GSSG, to its reduced form, GSH, consists of the modified glutathione reductase solution (0.25 ml; containing 3.1 mg of protein‡), GSSG (1.2×10^{-2} mol dm^{-3}) and Pt colloid§ (1 ml; 120 mg l^{-1}), under 1 atm of H_2. The Pt colloid serves as a catalyst for the reduction of the protein-bound bipyridinium relay by hydrogen.[8] The solution is stirred at room temperature. Fig. 1 presents the evolution of GSH in the system at various time intervals.¶ It can be seen that the reaction proceeds readily under these conditions. Control experiments reveal that no GSH is detectable in the system in the absence of the modified glutathione reductase or the Pt colloid, nor when native glutathione reductase is used in the absence of the electron relay. Furthermore, virtually no GSH is detected when the free bipyridinium salt is added to a system in which the native glutathione reductase is present. The presence of the modified enzyme is a prerequisite for the reduction process to occur.

We therefore deduce that the reaction proceeds through the catalytic cycle presented in Fig. 2. Molecular hydrogen reduces, in the presence of the Pt colloid, the protein-bound bipyridinium relay. The latter acts as a conductive component, transferring the electrons to the active site of the enzyme, where reduction of GSSG takes place. The overall reaction corresponds to the reduction of glutathione by hydrogen, as summarized by eqn. (1).

$$H_2 + GSSG \rightarrow 2GSH \quad (1)$$

In conclusion, electrical wiring of glutathione reductase provides us with the means for the chemical reduction of GSSG to GSH with hydrogen as the reductant, at very mild conditions. We believe that the approach of chemically modifying redox enzymes by electrically conductive elements is of general application in tailoring novel enzymes as catalysts for biocatalysed transformations.

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† Protein concentration is assayed by the biuret method.

‡ The concentration of the protein-bound bipyridinium salt in the solution is estimated in the following way: the sample (0.2 ml) is diluted with phosphate buffer (0.8 ml) 0.1 mol dm^{-3}, pH 7.5. In this solution sodium dithionite (5 mg) is dissolved. The absorption at λ = 602 nm is measured after 5 min, (ε = 11800 dm^3 mol^{-1} cm^{-1}). The bipyridinium-modified enzyme exhibits 5% of the activity of the native enzyme, as assayed by the reduction of GSSG of NADPH.[6] Nevertheless, this standard assay does not reflect the activity of the wired enzyme. Decreasing the loading of the bipyridinium component on the protein results in an increase in the activity determined by the standard assay, but a concomitant decrease in the effectiveness of the wired mechanism. This may suggest that the binding of NADPH in the protein is inhibited in the modified enzyme.

§ Pt colloid is prepared according to published methods, using citrate as the stabilizer.[7]

¶ The concentration of GSH is determined by Ellman's method.[9] Phosphate buffer (1.9 ml) 0.1 mol dm^{-3}, pH 7.6 and 5,5'-dithiobis(2-nitrobenzoic acid) BIS, reagent (0.1 ml; 1.6 mg BIS ml in 0.1 mol dm^{-3} phosphate buffer at pH 7.0) are added to the reaction sample (0.1 ml). Absorbance at λ = 412 nm (ε = 13600 dm^3 cm^{-1}) is measured after 2 min.