# NADPH:protochlorophyllide oxidoreductase from *Synechocystis*: overexpression, purification and preliminary characterisation

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**Abstract NADPH:protochlorophyllide oxidoreductase (POR) catalyses the light-dependent reduction of protochlorophyllide to chlorophyllide, a key regulatory reaction in the chlorophyll biosynthetic pathway. POR from the cyanobacterium *Synechocystis* has been overproduced in *Escherichia coli* with a hexahistidine tag at the N-terminus. This enzyme ($His_6$-POR) has been purified to homogeneity and a preliminary characterisation of its kinetic and substrate binding properties is presented. Chemical modification experiments have been used to demonstrate inhibition of POR activity by the thiol-specific reagent *N*-ethyl maleimide. Substrate protection experiments reveal that the modified Cys residues are involved in either substrate binding or catalysis.** **© 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

*Key words:* NADPH:protochlorophyllide oxidoreductase; Chlorophyll biosynthesis; Light-dependent; Chemical modification; *Synechocystis*

## 1. Introduction

In angiosperms, the reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) is catalysed by NADPH:protochlorophyllide oxidoreductase (POR, EC 1.3.1.33) [1]. Light is essential for the activity of POR and consequently this reaction is an important regulatory step in the chlorophyll biosynthetic pathway. In addition to POR, non-flowering land plants, algae and cyanobacteria possess a light-independent Pchlide reductase, consisting of three separate subunits [2]. In these organisms Chlide synthesis can occur in the dark and it appears that the activity of the light-dependent enzyme is important for maximum chlorophyll synthesis [3–5].

In higher plants, there are two POR isoforms, termed PORA and PORB, which are differentially regulated. PORA accumulates to high levels in the dark and is rapidly degraded upon illumination whereas PORB remains at a constant level throughout illumination [6]. In contrast, some organisms that can form chlorophyll in the dark, such as *Chlamydomonas rheinhardtii* [7] and *Synechocystis* sp. PCC6803 [8], contain only a single POR-encoding gene. Furthermore, PORs from cyanobacteria do not accumulate when they are grown in the dark and are only ever present at very low levels [9], in both plasma and thylakoid membrane fractions [10]. This is probably due to the fact that these organisms possess the light-independent reductase in addition to POR, which prevents the accumulation of Pchlide and the ternary complex. This low level of enzyme together with the presence of interfering pigments has previously made PORs from cyanobacteria much more difficult to study than the enzymes from higher plants.

Cyanobacteria are generally good model organisms for the study of chlorophyll biosynthesis as they share many of the features of higher plants whilst retaining the advantages of being prokaryotes. In recent years, heterologous expression of *Synechocystis* chlorophyll biosynthetic enzymes has been invaluable for the study of several steps in the pathway. Complementation of bacteriochlorophyll-minus *Rhodobacter* mutants led to the identification of *Synechocystis chl* genes encoding magnesium protoporphyrin IX methyl transferase [11], the light-dependent protochlorophyllide oxidoreductase [12] and the geranylgeranyl chlorophyll reductase [13]. Expression of the *chlH*, *I* and *D* genes in *Escherichia coli* led to the characterisation of the steady state kinetics of magnesium chelatase from *Synechocystis* [14–16]. Similarly, Oster et al. [17] successfully expressed the *chlG* gene encoding chlorophyll synthetase in *E. coli*.

The reaction catalysed by POR has been studied extensively and a number of spectroscopic forms of bound Pchlide have been identified [18]. However, to date, the catalytic mechanism of POR has not been elucidated. Comparisons of the amino acid sequence of POR with other sequences in the database have indicated that it is a member of the short-chain dehydrogenase family of enzymes [19,20] and mutational analysis of two residues conserved in all such dehydrogenases demonstrated that they are crucial for POR activity [19]. Detailed kinetic and structural studies are now necessary to further our understanding of the catalytic mechanism. However, an abundant source of pure enzyme is needed for this work.

Our recent success overexpressing POR from pea in *E. coli* as a fusion with maltose binding protein (MBP) and the subsequent demonstration of activity in vitro provided an excellent opportunity to study the structure and activity of this enzyme [21]. More recently, this system has been used to overexpress PORs from *Synechocystis* [22] and barley [23]. The main drawback of this approach is that it has not yet been possible to cleave these fusion proteins and so all experiments have been conducted with the 40 kDa MBP fused to the N-terminus of the enzyme. In the present paper, we report the

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*Abbreviations:* POR, NADPH:protochlorophyllide oxidoreductase; Pchlide, protochlorophyllide; Chlide, chlorophyllide; $His_6$-POR, hexahistidine-tagged POR; IPTG, isopropyl β-D-thiogalactoside; DTT, dithiothreitol; NEM, *N*-ethyl maleimide; NPM, *N*-phenyl maleimide

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high-level synthesis of POR from *Synechocystis* sp. PCC 6803 in *E. coli* with a small N-terminal histidine-tag. The presence of the histidine tag has allowed a single-step purification on a $Ni^{2+}$–Sepharose affinity column yielding large amounts of protein for characterisation of the kinetic and substrate binding properties of this enzyme.

## 2. Materials and methods

### 2.1. Construction of expression plasmid

The DNA encoding POR was amplified from *Synechocystis* sp. PCC 6803 genomic DNA by PCR using two sets of oligonucleotide primers specific to the 5′ and 3′ ends of the coding region according to the published sequence [12]. The forward primer, PETF (5′-GTGCATATGGAGACCGCGGCTCCGGCC-3′), introduced a *Nde*I site and the reverse primer, PETR (5′-GGGGGATCCCACTTTAGGCCAAACCAACAAGC-3′), introduced a *Bam*HI site. The resulting PCR product was treated with *Nde*I and *Bam*HI and cloned into the *Nde*I/*Bam*HI sites of pET9-His. This plasmid is a derivative of pET-9a (Novagen) in which the *Xba*I–*Nde*I fragment from pET-14b (Novagen), containing DNA encoding the N-terminal histidine tag, has been introduced. *E. coli* BL21 cells were transformed with the recombinant plasmid (pET9-His-POR) and a clone over-expressing hexahistidine-tagged POR ($His_6$-POR) on induction with 0.4 mM isopropyl β-D-thiogalactoside (IPTG) was isolated. The entire insert of the plasmid was sequenced using the ABI PRISM Dye Terminator Sequencing kit with Amplitaq DNA polymerase, FS (Perkin-Elmer).

### 2.2. Expression and purification of $His_6$-POR

*E. coli* BL21(pET9-His-POR) cells were grown in LB medium containing 25 μg/ml neomycin in 2 l flasks at 25°C and 250 rpm. When the cells reached an optical density of 0.5 at 600 nm the expression of $His_6$-POR was induced by the addition of IPTG to a final concentration of 0.4 mM. The cells were then grown for a further 2 h before harvesting by centrifugation. The cell pellet was resuspended in chilled binding buffer (500 mM NaCl, 20% glycerol, 5 mM imidazole, 0.1% Triton X-100, 20 mM Tris–HCl, pH 7.5) and disrupted by sonication for 3 min. Cell debris was removed by centrifugation and the supernatant loaded onto a 2.0×6.0 cm column packed with Chelating Sepharose Fast-Flow resin (Pharmacia Biotech) charged with $Ni^{2+}$ and pre-equilibrated with binding buffer. The column was washed with 10 column volumes of binding buffer and five column volumes of binding buffer containing 100 mM imidazole to remove any loosely bound contaminants. The recombinant protein was eluted with binding buffer containing 250 mM imidazole.

### 2.3. Protein determination and SDS–polyacrylamide gel electrophoresis

Protein concentrations were determined using the Bio-Rad *DC* protein assay with bovine serum albumin as standard. The expression level in *E. coli* and purification of $His_6$-POR was examined by SDS–polyacrylamide gel electrophoresis according to the method of Laemmli [24]. The proteins were separated by electrophoresis through a 12% polyacrylamide gel and visualised by staining with Coomassie brilliant blue R250.

### 2.4. Activity measurements

Pchlide was purified from *Rhodobacter capsulatus* ZY5 cultures as described previously [21]. The concentrations of the different pigments were determined using the following extinction coefficients in aqueous solution: NADPH, 6.22 $mM^{-1}$ $cm^{-1}$ at 340 nm; Pchlide, 23.95 $mM^{-1}$ $cm^{-1}$ at 630 nm [25,26]; and Chlide, 69.95 $mM^{-1}$ $cm^{-1}$ at 670 nm [26,27]. A Shimadzu 2101 split-beam UV–visible spectrophotometer was used to measure the initial rates of Chlide production over a range of substrate concentrations during continuous illumination of 0.04 μM $His_6$-POR in assay buffer (0.1% Triton X-100, 0.1% (v/v) β-mercaptoethanol, 50 mM Tris–HCl, pH 7.5). A Schott KL1500 electronic cold light source with a blue insert filter and a short-pass interference filter (Ealing 35-5362) provided illumination (approximately 1500 μmol $m^{-2}s^{-1}$) in the Soret region of the Pchlide absorption spectrum. A red cut-on filter (Schott RG 610) which blocks transmission of light below 600 m was used to protect the photomultiplier detector from the actinic light. The apparent $K_m$ and $V_{max}$ values were obtained by fitting the initial rates of Chlide synthesis against the concentrations of each substrate to the following equation:

$$v = (V_{max}^{app}[S])/(K_m^{app} + [S]) \quad (1)$$

where $v$ is the initial rate, [S] is the substrate concentration, $V_{max}^{app}$ is the initial rate achieved as [S] approaches infinity and $K_m^{app}$ is the apparent value of [S] giving $V_{max}/2$. Data were fitted using the Sigma Plot program (SPSS Inc.).

### 2.5. Fluorimetric binding analysis

The fluorescence enhancement of NADPH on binding to $His_6$-POR was used to determine the apparent dissociation constant of NADPH to the enzyme. Fluorescence titrations were performed in a SPEX FluoroLog spectrofluorimeter at 20°C by making microlitre additions to 3 ml of 0.04 μM $His_6$-POR in 0.1% (v/v) Triton X-100, 0.1% (v/v) β-mercaptoethanol, 50 mM Tris–HCl, pH 7.5. The maximum volume change resulting from the addition of NADPH was less than 2%. An excitation wavelength of 340 nm was used and the emission intensity measured at 460 nm. Additions of NADPH were made both in the presence and absence of $His_6$-POR.

The apparent $K_d$ values were obtained by fitting the fluorescence changes against the concentration of NADPH using the following equation:

$$\Delta F = \Delta F_{max}^{app}[NADPH]/K_d^{app} + [NADPH] \quad (2)$$

where $\Delta F$ is the enhancement of fluorescence upon binding to POR, $\Delta F_{max}^{app}$ is the apparent maximum change in fluorescence and $K_d^{app}$ is the apparent dissociation constant.

The data were fitted and standard errors calculated by non-linear regression analysis using the Sigma Plot program (SPSS Inc).

### 2.6. Inhibition of POR activity by N-ethyl maleimide (NEM)

A 100 μl solution of 11.6 μM $His_6$-POR in 0.1% (v/v) Triton X-100, 50 mM Tris–HCl, pH 7.5 was incubated with different concentrations of NEM at 25°C. The chemical modification reaction was stopped after 30 min by the addition of 1 μl β-mercaptoethanol. The entire reaction mixture was then added to 900 μl of 0.1% (v/v) Triton X-100, 50 mM Tris–HCl, pH 7.5 containing NADPH and Pchlide. The final concentrations of these two substrates in the assay mixture were 20 and 8 μM respectively. The enzyme activity was then measured under continuous illumination as described previously.

To test whether the presence of substrates protected the enzyme from inhibition by NEM, the enzyme was incubated with 500 μM NEM in the presence of either 8 μM Pchlide or 10 μM NADPH or both substrates for 30 min. The reaction was then stopped and the activity measured under the same conditions as described above.

## 3. Results

### 3.1. Expression and purification of $His_6$-POR

The pET9-His expression vector was used to express *Synechocystis* POR fused to a hexahistidine affinity tag, resulting in the incorporation of an extra 20 amino acids at the N-terminus of the enzyme. Analysis of the sequencing data confirmed the insertion of DNA encoding *Synechocystis* POR into the expression vector. Comparison of the nucleotide sequence with the two sequences of *Synechocystis por* available in the database indicated that the sequence was identical to that published by Kaneko et al. [28] but differed from the sequence published by Suzuki and Bauer [12] by virtue of an exchange of a cytosine and a guanine. The latter nucleotide sequence encodes an arginine residue (codon CGT) at position 19 whereas the sequence we obtained codes for an alanine residue (codon GCT) at this position. The *por*s from all other organisms sequenced to date encode an alanine at this position and so it is likely that the sequence we obtained is correct.

The expression of $His_6$-POR in *E. coli* was monitored by SDS–PAGE. After induction with IPTG, a band with a mo-

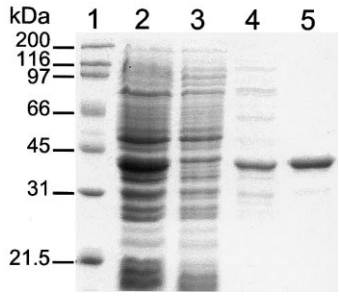


Fig. 1. SDS–PAGE analysis of $His_6$-POR. Coomassie blue-stained SDS–PAGE gel showing the purification of $His_6$-POR on a $Ni^{2+}$-affinity column. Lane 1, molecular-mass markers (myosin, 200 kDa; β-galactosidase, 116 kDa; phosphorylase *b*, 97.4 kDa; BSA, 66.2 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 31 kDa and trypsin inhibitor, 21.5 kDa); lane 2, cytoplasmic proteins released from the induced cells by sonication; lane 3, proteins not bound to column; lane 4, protein washed off column with 100 mM imidazole in binding buffer and lane 5, $His_6$-POR eluted with 250 mM imidazole in binding buffer.

lecular mass of approximately 38 kDa appeared which corresponds to the predicted molecular mass of $His_6$-POR (37.7 kDa). The protein was estimated to constitute 20% of the total soluble cell protein. Crude extracts from induced and non-induced cells were assayed for POR activity and Chlide formation was detected only in the induced samples. After a single step affinity chromatography purification from crude *E. coli* cell extract, $His_6$-POR was estimated to be greater than 95% pure (Fig. 1). Approximately 60 mg of purified protein was routinely obtained from 1 l of starting culture.

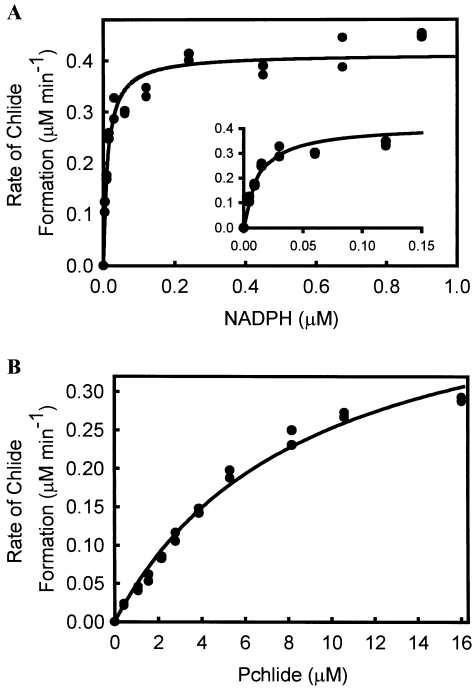


Fig. 2. Graphical representation of the data used to determine the kinetic parameters. Rate of Chlide synthesis catalysed by 0.04 μM $His_6$-POR at A: a fixed concentration of Pchlide (14.2 μM) and varying NADPH concentration and B: a fixed concentration of NADPH (3 μM) and varying Pchlide concentration. The points represent the experimental data and the lines represent the predicted values obtained by fitting the data points to Eq. 1 by non-linear regression analysis (see Section 2).

### 3.2. Determination of the kinetic parameters

Initial experiments indicated that purified $His_6$-POR catalysed the reduction of Pchlide to Chlide upon illumination and performed multiple turnovers. However, the enzyme was only active in the presence of reducing agents such as β-mercaptoethanol or dithiothreitol (DTT). To determine the kinetic parameters for $His_6$-POR it was necessary to measure the initial rate of Chlide formation over a range of NADPH and Pchlide concentrations. The dependence of the initial rate on substrate concentration followed Michaelis–Menten kinetics, shown graphically in Fig. 2. The $V_{max}$ was determined to be $0.42 \pm 0.01$ μM $min^{-1}$ in the presence of 14.2 μM Pchlide and $0.47 \pm 0.02$ μM $min^{-1}$ in the presence of 3 μM NADPH. The apparent $K_m$ for Pchlide was calculated to be $8.6 \pm 0.9$ μM and the apparent $K_m$ for NADPH was $0.012 \pm 0.002$ μM.

### 3.3. Analysis of dinucleotide binding

The fluorescence of NADPH becomes significantly enhanced upon binding to $His_6$-POR and the fluorescence maximum also shifts to a shorter wavelength (Fig. 3A). NADPH was excited at 340 nm and the fluorescence emission spectrum recorded between 400 and 600 nm. Under these conditions the NADPH fluorescence increased linearly with concentration indicating that the inner filter effects were negligible. When $His_6$-POR was included in the mixture, difference spectra in-

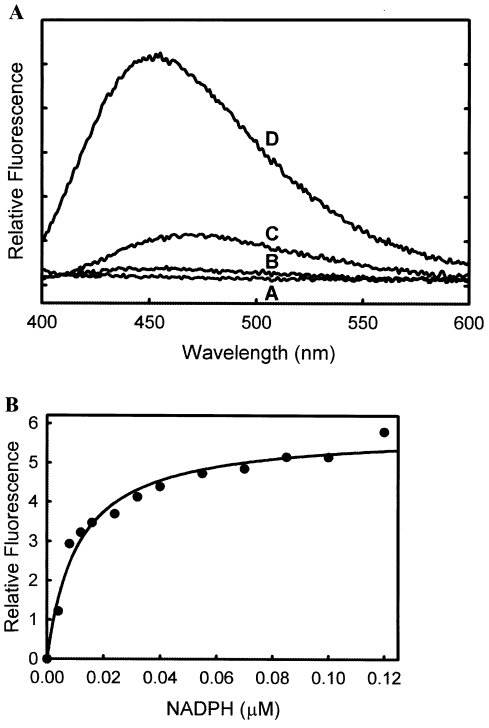


Fig. 3. Enhancement of NADPH fluorescence upon binding to $His_6$-POR. A: Fluorescence emission spectra of free and POR-bound NADPH. An excitation wavelength of 370 nm was used with excitation–emmission slits of 9 nm. Spectra of the following samples were recorded: **A**, assay buffer; **B**, assay buffer containing 0.5 μM $His_6$-POR; **C**, assay buffer containing 1 μM NADPH and **D**, assay buffer containing 1 μM NADPH and 0.5 μM $His_6$-POR. B: Fluorescence titration of 0.04 μM $His_6$-POR with NADPH. Excitation wavelength was 340 nm and emission wavelength was 460 nm. The points represent the experimental data and the line represents the predicted values obtained by fitting the data points to Eq. 2 by non-linear regression analysis (see Section 2).

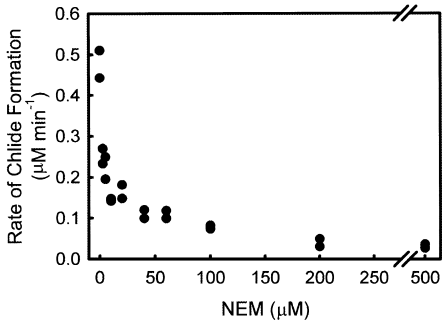


Fig. 4. Inhibition of $His_6$-POR activity by NEM. 11.6 μM $His_6$-POR was incubated with varying concentrations of NEM for 30 min at 25°C. The reaction was stopped by addition of 1% (v/v) β-mercaptoethanol prior to making a 1/10 dilution of the enzyme mix and measuring the rate of light-dependent Chlide production in the presence of 20 μM NADPH and 8 μM Pchlide.

dicated the biggest change in fluorescence was observed at 454 nm. A titration curve showing the fluorescence enhancement upon binding of NADPH to 0.04 μM $His_6$-POR is given in Fig. 3B. Under these conditions the $K_d^{app}$ for NADPH was calculated to be $0.011 \pm 0.002$ μM.

Similar experiments to those described above were performed with NADH. However no enhancement in NADH fluorescence was observed when $His_6$-POR was included in the mixture (data not shown), indicating that NADH is unable to bind to the enzyme.

To determine if Pchlide has an effect on NADPH binding, titrations were repeated in the presence and absence of 2 μM Pchlide (data not shown). As the reaction catalysed by POR is light-dependent, the excitation wavelength chosen was 310 nm, as far away as possible from the Soret region of the absorption spectrum of Pchlide. Under these conditions spectroscopic measurements indicated that the enzyme had not turned over. The presence of Pchlide was found not to affect the binding of NADPH to $His_6$-POR.

### 3.4. Inhibition of $His_6$-POR by NEM

Treatment with NEM was found to inhibit $His_6$-POR activity and was dependent on the concentration of NEM used (Fig. 4). At an NEM concentration of 500 μM, approximately 13% of the activity remained after 30 min incubation. The experiment was then repeated in the presence of Pchlide, NADPH or both substrates (Table 1). $His_6$-POR which had been pre-incubated with Pchlide showed little difference to unprotected $His_6$-POR, indicating that Pchlide on its own offers no protection against NEM treatment. However, inclusion of NADPH prior to NEM treatment allows over half of the activity to be retained while pre-incubation with both substrates provides even greater protection, reducing the level of inhibition significantly.

Table 1
Inhibition of $His_6$-POR activity on treatment with NEM in the presence or absence of substrates

| Preincubation before NEM treatment | Activity retained after NEM treatment (percent of activity of untreated $His_6$-POR) |
|---|---|
| $His_6$-POR | 13.2 |
| $His_6$-POR+Pchlide | 13.9 |
| $His_6$-POR+NADPH | 59.6 |
| $His_6$-POR+NADPH+Pchlide | 80.8 |

$His_6$-POR was incubated for 30 min with or without 8 μM Pchlide and/or 10 μM NADPH prior to treatment with 500 μM NEM for 30 min at 25°C. After NEM treatment, the rate of Chlide formation was measured as described in Section 2.

## 4. Discussion

In order to further our understanding of the catalytic mechanism of POR we have overexpressed the *Synechocystis* enzyme with a hexahistidine tag in *E. coli* using the pET9-His expression vector. $His_6$-POR was produced in large quantities as a soluble protein that could be purified by a single-step purification procedure on a $Ni^{2+}$–Sepharose affinity column. This was sufficient to produce near homogeneous protein and the major band that was observed on a Coomassie blue-stained gel was the size expected for *Synechocystis* POR with an N-terminal hexahistidine tag. The purified enzyme is catalytically active and has the advantage of incorporating a much smaller tag than the MBP–POR fusion proteins described previously [21–23]. This has enabled us to carry out a preliminary kinetic and binding characterisation of a near-native POR.

The $K_m^{app}$ of $His_6$-POR for NADPH was calculated to be 0.012 μM. This is substantially lower than values previously obtained for PORs from *Scenedesmus oliquus* (4.2 μM) [29], barley etioplast membranes (35 μM) [1] and pea MBP–POR (8.67 μM) [21]. The $K_m^{app}$ of *Synechocystis* $His_6$-POR (8.6 μM) for Pchlide is over 10-fold higher than the $K_m^{app}$s reported for PORs from *Scenedesmus* (0.19 μM) [29], barley etioplast membranes (0.46 μM) [1] and MBP–POR from pea (0.27 μM) [21]. The specific activity of *Synechocystis* $His_6$-POR was determined to be 270 nmol $min^{-1}$ $mg^{-1}$ in the presence of 14.2 μM Pchlide and 309 nmol $min^{-1}$ $mg^{-1}$ in the presence of 3 μM NADPH. The latter value is likely to be more accurate as this was determined at a saturating NADPH concentration whereas it was not possible to use a saturating concentration of Pchlide due to the very high $K_m^{app}$ for this substrate. Both specific activity values are substantially higher than the values obtained previously for purified pea and *Synechocystis* MBP–POR fusion proteins (20.6 nmol $min^{-1}$ $mg^{-1}$ and 11.2 nmol $min^{-1}$ $mg^{-1}$, respectively) [21,22]. The presence of the large MBP tag and/or differences in assay conditions may give rise to some of the variations. In the case of pea MBP–POR, the light intensity used was much lower than the light intensity used in the experiments described in this paper.

The enhancement in NADPH fluorescence observed upon binding to $His_6$-POR has allowed the $K_d^{app}$ for NADPH to be determined. The value obtained (0.011 μM) indicates that NADPH binds very tightly to the enzyme. The presence or absence of Pchlide has no effect on the binding parameters of NADPH, indicating that it is not necessary for Pchlide to be present before NADPH binds to POR. This result suggests that there is either ordered binding or that independent binding sites exist.

POR has previously been shown to be a member of the family of short-chain dehydrogenases, [19,20] which includes both NADPH and NADH utilising enzymes. Only very small changes in structure of the enzymes account for the different specificities for these two cofactors. Previous experiments have shown that POR would not utilise NADH [30]. Our experiments show that NADH does not bind to $His_6$-POR, despite

its similarity to NADPH. The nucleotide binding experiments described in this paper are the first of this kind to be performed on POR from any source and will be critical for the future examination of site-directed mutants of the cofactor binding site.

Kinetic analysis of the $His_6$-POR showed that the enzyme was active only in the presence of β-mercaptoethanol or DTT, perhaps indicating that a reactive thiol group is involved in either catalysis or substrate binding. This is supported by the fact that NEM inhibits the activity of $His_6$-POR and is in agreement with the findings of Griffiths on the barley enzyme [27]. Pchlide on its own offered no protection to modification whereas incubation with NADPH prior to NEM treatment resulted in retention of almost 60% of activity. Furthermore, pre-incubation with both substrates offered the greatest protection with over 80% of POR activity being retained. Previously, $^3$H-labelled *N*-phenyl maleimide (NPM) was used to label thiol groups exposed following illumination of the barley POR-NADPH-Pchlide photoactive ternary complex [31]. Subsequently, Cys-296 in POR from wheat (equivalent to Cys-222 in *Synechocystis* POR) was shown to be protected from modification by NPM in the presence of Pchlide and NADPH, and was suggested to be involved in substrate binding or catalysis [32]. Three cysteine residues are absolutely conserved among all known derived amino acid sequences of PORs. Sequence comparisons with other members of the short-chain dehydrogenase family indicate that Cys-33 is located within the nucleotide binding pocket and therefore this residue may be protected from chemical modification by bound NADPH. Another cysteine residue (Cys-195) is located in close proximity to the proposed active site residues, Tyr-189 and Lys-193, whilst the third conserved cysteine residue (Cys-222) may also be located in or near the active site [32]. Hence, in our experiments, Cys-195 and/or Cys-222 may be protected from NEM inhibition by pre-incubation with Pchlide, but only in the presence of NADPH. The fact that Pchlide on its own is unable to protect $His_6$-POR from modification by NEM may imply that Pchlide can only bind to the enzyme when NADPH is present (i.e. the order of binding is NADPH first followed by Pchlide). Our results do not exclude the possibility that Pchlide may bind to POR in the absence of NADPH with the enzyme remaining susceptible to inhibition by NEM. In this case, binding of NADPH to POR:Pchlide may induce a conformational change leading to increased protection of susceptible cysteine residues.

In conclusion, we have succeeded in producing large quantities of soluble His-tagged POR from *Synechocystis* in *E. coli*. $His_6$-POR has been purified by a single affinity chromatography step and some of the kinetic and substrate binding properties of the enzyme have been characterised. This system provides an excellent model for future analysis of mutant enzymes that will facilitate determination of the complete catalytic reaction mechanism of this unique and very important enzyme.

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