Supplementary Data

METHODS

Immunodetection of His-tagged PfENR

The protein bands run on SDS/PAGE (10%) were transferred onto PVDF membranes (Immobilon, Millipore) using Pharmacia LKB mutiphor II semi-dry Western-blot apparatus. The presence of His-tagged ENR was detected using mouse anti-His antibody (Novagen, Madison, U.S.A.) as the primary antibody and goat anti-mouse antibody conjugated to HRP (Bangalore Genei, Bangalore, India) as the secondary antibody. The blot was developed using 3-amino-9-ethylcarbazole (AEC) and hydrogen peroxide (Figure 1).

Gel-filtration analyses

Purified PfENR (2 mg/ml) was injected onto a Superdex™200 HR10×300mm column (Amersham Biosciences) equilibrated in 20 mM Tris/HCl, 150 mM NaCl (pH 7.4), connected to an ÄKTA™ design system at room temperature. The column flow rate was maintained at 0.3 ml/min. The column was calibrated using standards of known molecular mass (Figure 2).

CD studies

CD spectra were recorded on a Jasco J-715 spectropolarimeter at 20°C. ENR (5 μM) in 10 mM Tris/HCl, 150 mM NaCl (pH 7.4) was used during the measurements. Far-UV CD spectra were recorded from 200 to 250 nm at 0.5 nm intervals with a spectral bandwidth of 2 nm, using a 1 mm cuvette. Near-UV CD spectra were recorded from 250 nm to 350 nm using a 10 mm cuvette. Each scan represents an average of 6 scans.

RESULTS

CD analysis of the mutants

The CD spectra of the mutant PfENR variants and the wild-type enzyme were similar and showed no changes in the ellipticity in the secondary structural region (Figure 3). Moreover, the overall shape of the spectra remained the same. In the near-UV spectra, there are slight differences in the molar ellipticities of the wild type and F368I. These minor differences in the absorption can be attributed to the changes of the chromophoric amino acid with non-chromophoric residue, namely the replacement of phenylalanine (absorption maximum at 254—256 nm) with isoleucine. A217V, A217G, N218D and M281T gave tertiary CD spectra similar to the wild-type protein. Thus, the various amino acid substitutions had no effect on the overall folding of the resultant mutant proteins.

FIGURE LEGENDS

Figure 1 SDS/PAGE and Western-blot analysis of PfENR mutants

(**A**) SDS/PAGE (10% gel) showing the mobility of PfENR mutants. Lanes 1--5: A217V, A217G, N218D, M281T and F368I; lane 6: MBI, protein molecular mass marker. (**B**) Western-blot analysis of the ENR mutants. Lane 1--5: A217V, A217G, N218D, M281T and F368I; lane 6: Prestained protein molecular mass marker.

Figure 2 Gel filtration profile of wild-type and mutant ENR

Gel filtration profile of the wild-type (short dash), A217V (line), A217G (long dash), N218D (dotted), M281T (dash-dot) and F368I (dash-dot-dot) mutants. ENR elutes as a single peak at an elution volume of 7.6 ml on a Superdex-200 gel filtration column. Arrows show the elution volume of the standard molecular mass markers. The standards used were: lysozyme (14 kDa), carbonic anhydrase (29 kDa), hen egg ovalbumin (45 kDa), bovine serum albumin (66 kDa), aldolase (158 kDa).

Figure 3 CD spectrum analysis of wild-type, A217V, A217G, N218D, M281T and F368I mutants

(**A**) Far-UV spectra obtained for 6 μM protein in 10 mM Tris/HCl, 100 mM NaCl, pH 7.4 at 20º C from 200--260 nm using the 1 mm cuvette. (**B**) Near-UV spectra obtained for 6 μM protein in the same buffer from 230--350 nm at 20º C using the 10 mm cuvette. Wild-type (short dash), A217V (line), A217G (long dash), N218D (dotted), M281T (dash-dot) and F368I (dash-dot-dot) mutant.

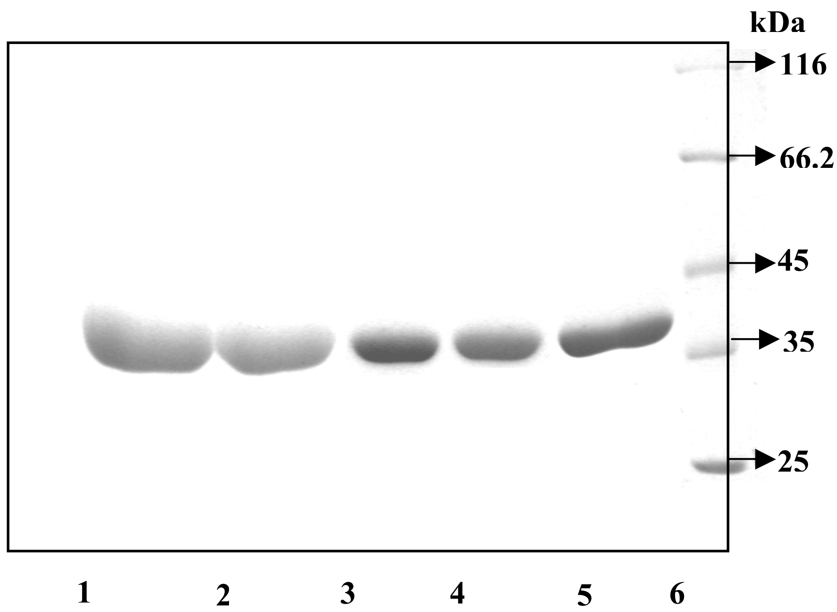

Figure 1A

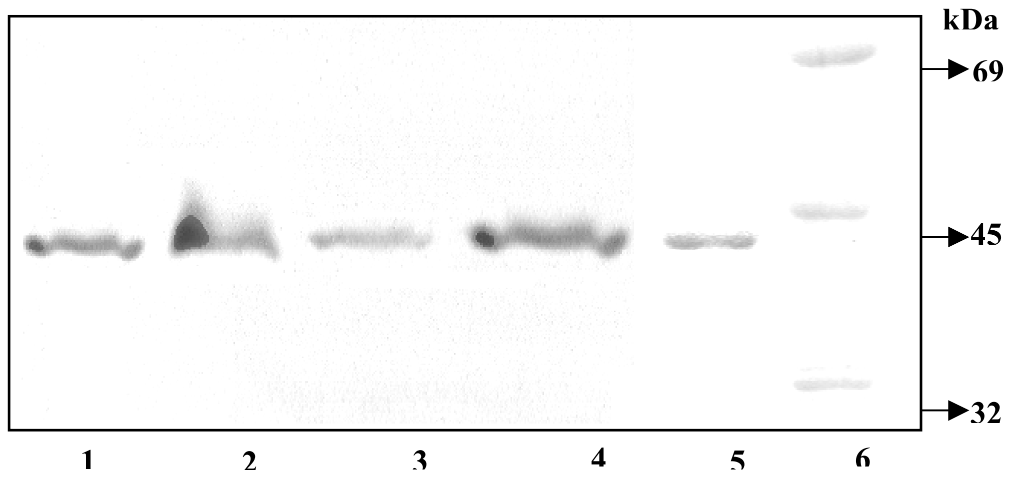

Figure 1B

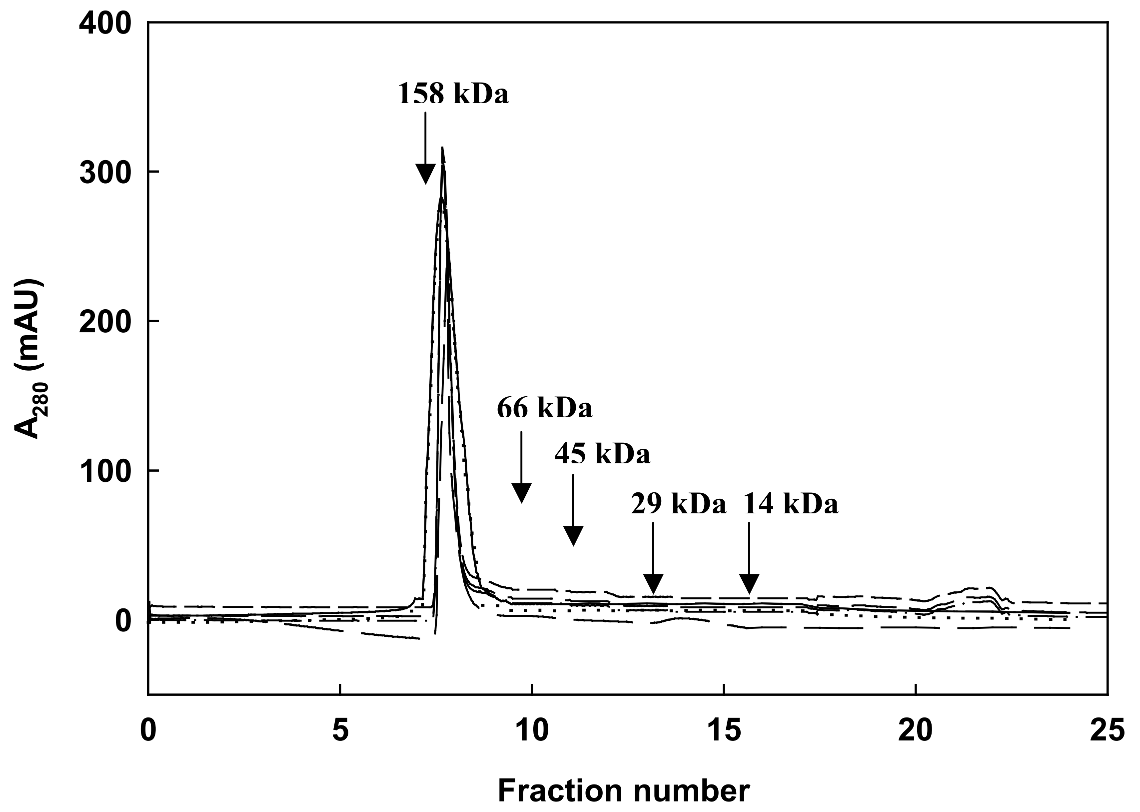

Figure 2

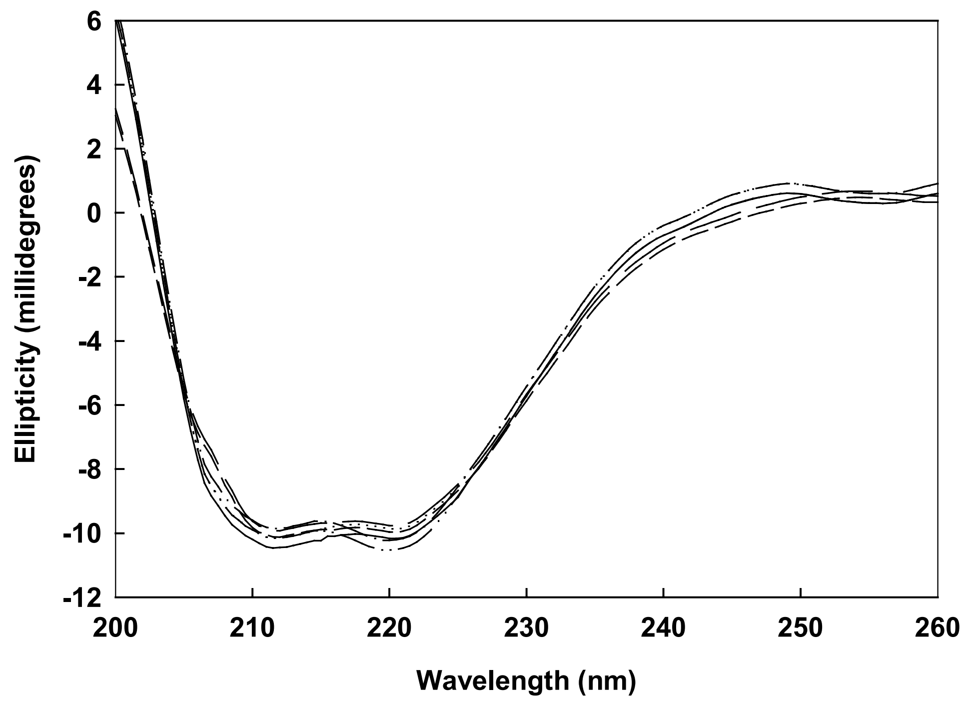

Figure 3A

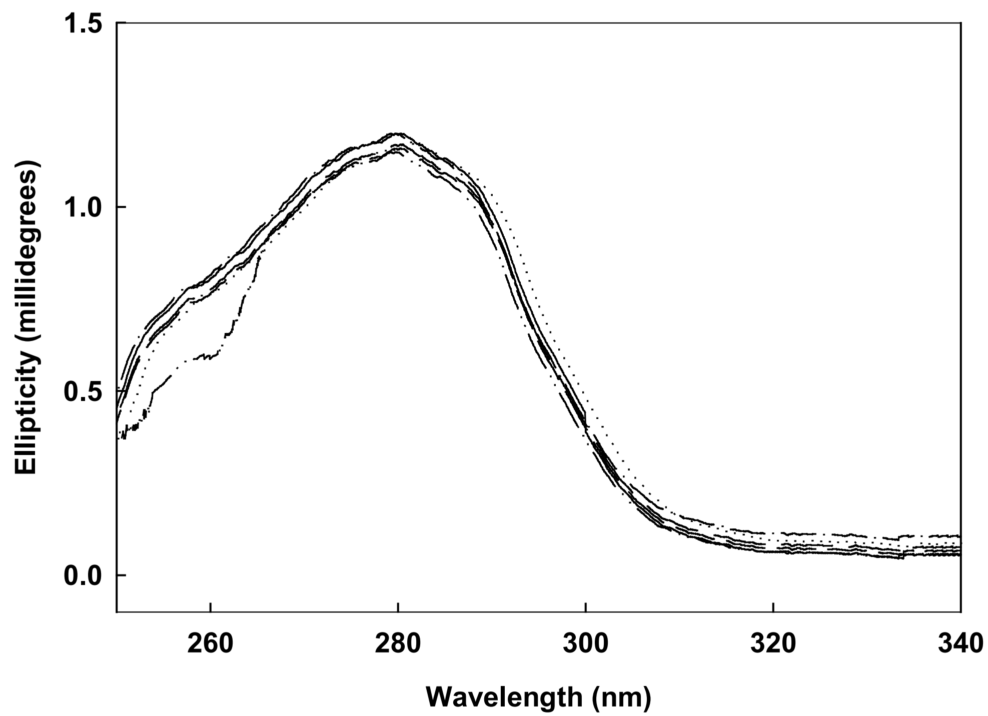

Figure 3B