However, when bands with PHGPx standards from human liver and placenta were detected by ion-exchange on DEAE-sephacel and Western blotting, the peroxidase activity in the membrane extract was less than 17% as observed from 19,671 Da Se-PHGPx. Two higher molecular weight bands (30 and 52 kDa) were detected, and these bands may be higher molecular weight PHGPx or alternatively may be non-specific interactions.

Human liver PHGPx was purified as described previously [11]. Anti-PHGPx (immun serum against pig heart PHGPx) was kindly supplied by Dr A. Roveri (University of Padova, Italy). Heparoyl-phospholipid (PLPC-OOH) was prepared from PLPC using lipoxidasase as described by Maiorino et al [12]. Pig blood was obtained fresh, and heparin (30U/ml) was used for anticoagulation. Erythrocyte ghost membranes were prepared [9] in the presence of 5mM EDTA and centrifuged at 10,000g for 30 min. The supernatant was dialysed overnight against 50mM Tris-HCl (pH 7.4) containing 5mM EDTA and one change of dialysis buffer. After centrifugation, the supernatant was used for PHGPx assay and immuno blotting. The peroxidase activity of erythrocytes on PLPC-OOH was measured directly by HPLC [14].

There are no reports about the presence of PHGPx in blood. However, when a whole blood sample was tested for PHGPx assay, it exhibited higher PHGPx-like activity than attributable to the effect of albumin and the activity of Hb and vitamin E (unpublished results). This PHGPx-like activity was traced by assaying the separated white cells and platelets. Neither white cells nor platelets exhibited PHGPx activity. Therefore, the unaccounted PHGPx-like activity was mainly in the red blood cells. The cell contents after lysis were fractionated by ion-exchange on DEAE-sephacel and affinity chromatography on bromosulphophthalein-glutathione-agarose, but no PHGPx activity was detected. When an erythrocyte membrane extract was tested for PHGPx activity, the specific activity was 5U/mg protein. Whether this activity was from the 19,671 Da Se-PHGPx, gene product of Gpx4 [15, 16] was further investigated by immuno blotting.

Pig heart PHGPx antibody recognizes PHGPx protein from most rat, pig and human tissues. Fig 1 shows a result of the analysis of pig erythrocyte membrane extracts with pig heart PHGPx antibody. Both PHGPx standards from human liver and placenta gave two clear bands with Mr~20 kDa. No corresponding 19,671 Da PHGPx band was detected in the erythrocyte membrane extracts. The detection limit of pure PHGPx was 1 ng (specific activity=336 U/mg [11]) in immuno blotting analysis, and the sample applied in lane 1 was 0.4 mg protein (PHGPx activity =5 nU/mg). Therefore the contribution of PHGPx activity in the membrane extract was less than 17% from 19,671 Da Se-PHGPx. Two higher molecular weight bands (30 and 52 kDa) were detected, and these bands may be higher molecular weight PHGPx or alternatively may be non-specific interactions.

References