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Halina M. Żbikowska, Barbara Wachowicz

ISOLATION AND PARTIAL PURIFICATION OF A GLUTATHIONE PEROXIDASE FROM PIG BLOOD PLATELETS

An enzyme, glutathione peroxidase from pig platelets by the method of Maddipati and Marnett (1987) was isolated and partially characterized. The purification procedure involved ammonium sulphate precipitation, hydrophobic chromatography on phenyl– Sepharose CL–4B and anion exchange chromatography. SDS–electrophoresis of the enzyme obtained from the final chromatographic step revealed a 23 KDa subunit. It was established that selenium -dependent GSH–Px in platelets was predominant and showed about 86% of the total glutathione peroxidase activity. Sodium selenite enhanced the activity of the enzyme whereas copper ions, N–ethylmaleimide and cisplatin were the inhibitors of the GSH–Px activity.

1. INTRODUCTION

Glutathione peroxidase (GSH–Px) activity was found in various mammalian tissues and cells [1, 2]. Although it has also been isolated from different sources [3, 4, 5] little data exist on platelet GSH–Px. Blood platelets contain a large amount of selenium (0.5–0.7 μg/g wet weight) [6, 7, 8] and the selenium-dependent glutathione peroxidase is more active in these cells than in red blood cells [1, 3, 9]. GSH–Px assay can be used for determination of selenium content in human beings and animals [1]. GSH–Px is involved in the metabolism of fatty acid hydroperoxides and cyclic endoperoxides formed respectively, via the lipoxygenase and the cyclooxygenase pathways [10, 11]. In view of the important role of GSH–Px in platelet metabolism we have undertaken isolation and purification of the enzyme from pig platelets to study its physical and kinetic properties. Our preliminary results are presented in this paper.

2. MATERIALS AND METHODS

The enzyme was isolated from washed pig blood platelets as described by Maddipati and Marnett [4] for the purfication of human plasma GSH–Px. All purfication steps were performed at 4°C. Crude extracts of the enzyme were obtained by three cycles of freezing (–20°C) and thawing (37°C) of pig platelets. Briefly, purfication procedure included precipitation of the platelet cytosol protein with ammonium sulfate (20–60% saturation). Ammonium sulfate fraction dissolved in buffer A (0.1 M Tris–HCl, 0.2 M ammonium sulphate, 5 mM EDTA, 1 mM GSH, pH 7.2) was then applied on the column with phenyl–Sepharose CL–4 B and washed extensively until the 280 nm absorbance of the eluate was equal to that of the buffer. Afterwards the column was subjected to a linear gradient elution between 0.1–0.01 M Tris–HCl, pH 7.2. Active fractions (2 ml) were pooled and concentrated (YM – 10 membrane); the buffer was changed to 0.2 M Tris–HCl, 5 mM EDTA, 1 mM GSH, pH 8.0 (buffer B). The concentrated peroxidase was then applied to a DEAE–Sephadex A–50 column. After washing the column with about 200 ml of buffer B the enzyme was eluted with a linear gradient, between 0.2 M–0.4 M Tris–HCl, pH 8.0, both containing 5 mM EDTA and 1 mM GSH. Peroxidase acive fractions (1.6 ml) were pooled and concentrated to about 5 ml. Glutathione peroxidase activity towards cumene and t–butyl hydroperoxides was measured as described by Paglia and Valentine in the presence of sodium selenite (100 μM), $CuSO_4$ (20 mM), NEM (100 mM), cisplatinum (30 μM) and compared with control [12]. Protein was estimated according to Lowry et al. [13]. SDS–PAGE was performed according to the procedure of Laemmli [14] using 14% acrylamide gel.

3. RESULTS AND DISCUSSION

An enzyme with a glutathione peroxidase activity was isolated from pig blood platelets and partially purfied. The enzyme was similar to GSH–Px obtained from human platelets by Ramos Martinez et al [3]. The typical elution pattern of the phenyl–Sepharose CL–4B column is presented in Fig. 1.

The profile shows only the gradient elution of the column. Hydrophobic interaction chromatography on phenyl–Sepharose CL–4B proved to be a key step that eliminated most of the contaminating protein.

Fig. 2 shows the anion exchange chromatography of concentrated active fractions on DEAE–Sephadex A–50. The peroxidase was eluted as a single peak which may suggest the absence of multiple forms of the platelet

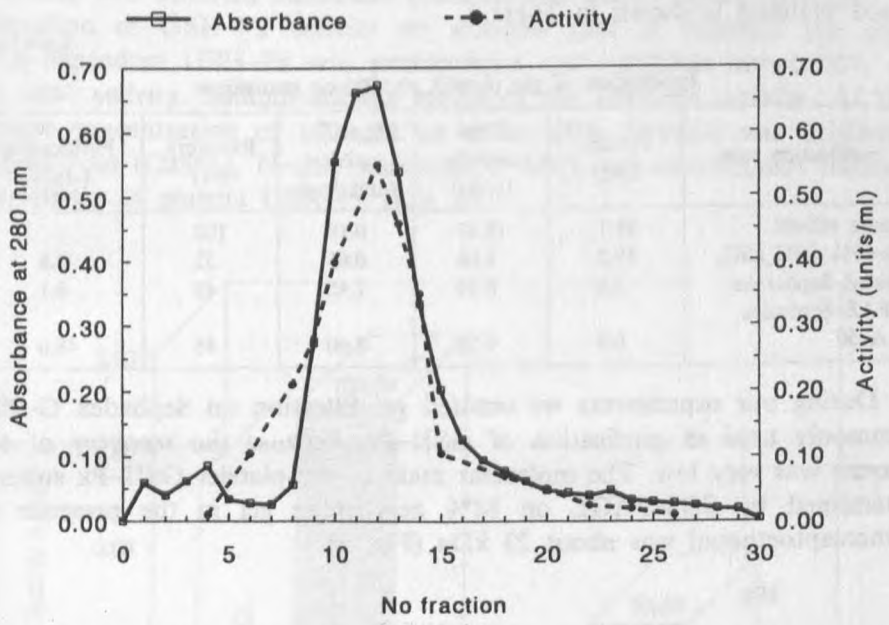

Fig. 1. Chromatography on phenyl–Sepharose CL–4B. The profile shows only the gradient elution of the column. Details are given under Materials and Methods

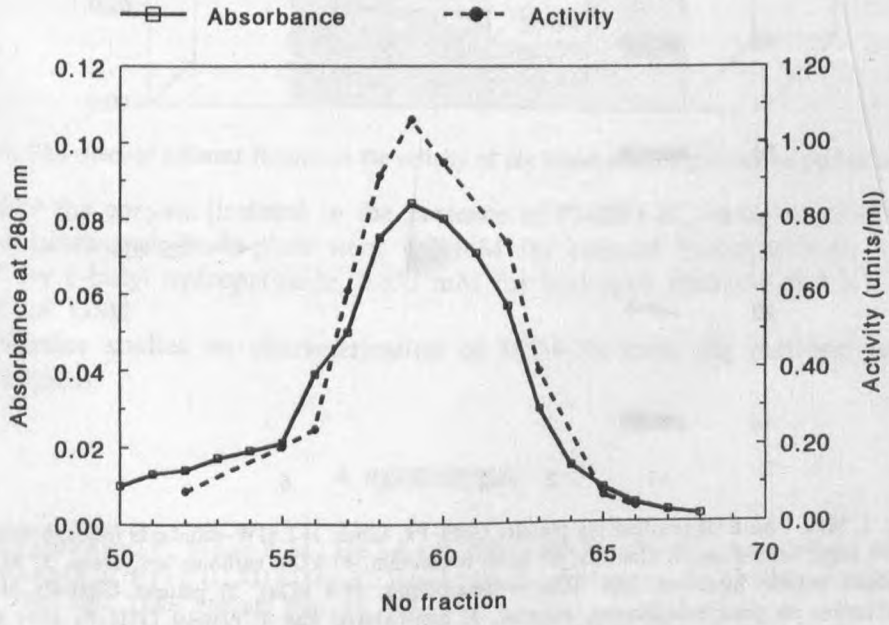

Fig. 2. Anion exchange chromatography on DEAE–Sephadex A–50

enzyme. A summary of the purification steps of GSH–Px activity from pig blood platelets is shown in Table 1.

Table 1

Purification of pig platelet glutathione peroxidase

Purification step	Protein (mg)	Total activity (units)	Specific activity (units/mg)	Recovery (%)	Purification (–fold)
Crude extract	91.1	16.61	0.18	100	
20–60% $(NH_4)_2SO_4$	13.2	8.64	0.65	52	3.6
Phenyl–Sepharose	5.6	8.14	1.46	49	8.1
DEAE–Sephadex A–50	0.9	7.73	8.60	46	48.0

During our experiments we omitted gel filtration on Sephadex G–200, commonly used in purfication of GSH–Px, because the recovery of the enzyme was very low. The molecular mass of the platelet GSH–Px subunit determined by SDS–PAGE on 14% acrylamide gel in the presence of β–mercaptoethanol was about 23 kDa (Fig. 3).

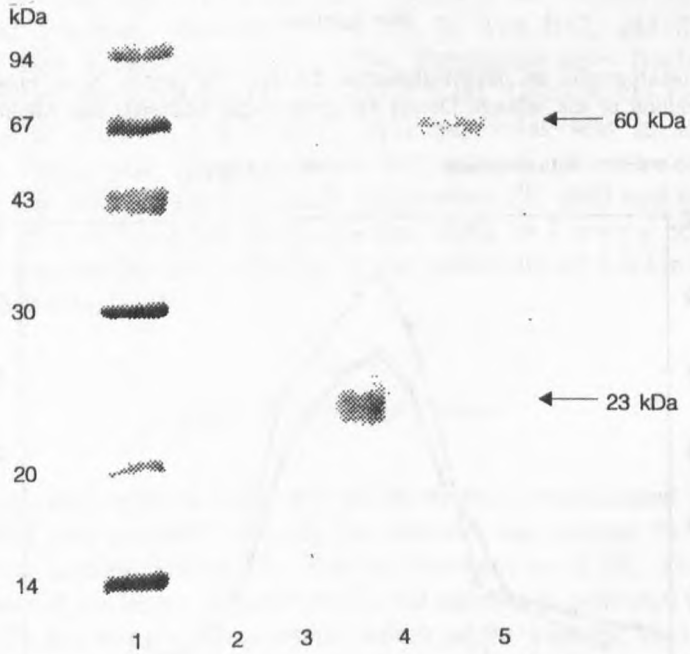

Fig. 3. SDS–PAGE of purified pig platelet GSH–Px. Lines: 1) LMW–standards (phosphorylase b, 94 kDa; bovine serum albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20.1 kDa; α–lactalbumin, 14.4 kDa); 2) platelet GSH–Px after purfication on phenyl–Sepharose, reduced, 3) non-reduced and 4) platelet GSH–Px after the last step purfication, reduced, 5) non-reduced

Using two different substrates (cumene and t-butyl hydroperoxides) in estimation of GSH–Px activity we assessed that in platelets the selenium–dependent GSH–Px was predominant and exhibited about 86% of the total activity. Sodium selenite enhanced the GSH–Px activity. At the selenite concentration of 100 μM an about 20% increase was achieved. Copper ions ($CuSO_4$), N–ethylmaleimide (NEM) and cis–platinum reduced the activity of platelet GSH–Px (Fig. 4).

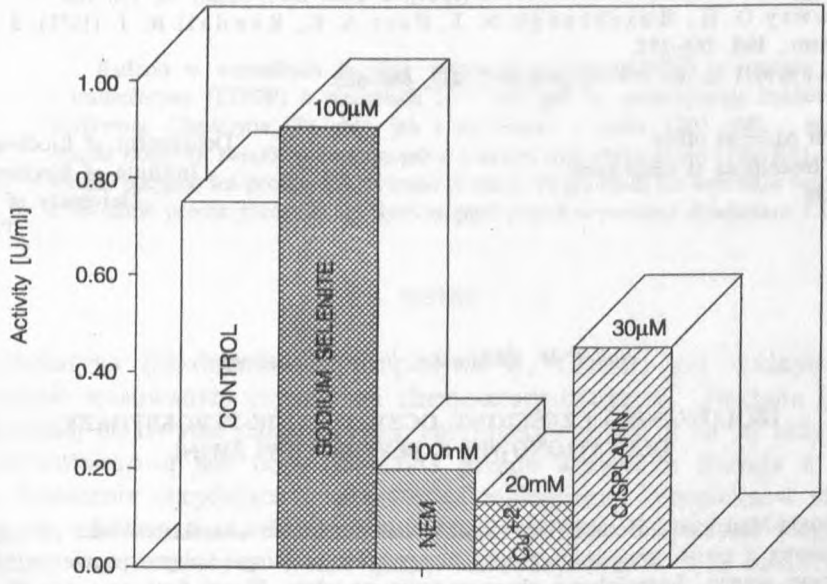

Fig. 4. The effect of different factors on the activity of pig blood platelet glutathione peroxidase

For the enzyme (isolated in the presence of PMSF) K_m value calculated from Lineweaver-Burk plots were: 5.9 mM for cumene hydroperoxide, 10 mM for t–butyl hydroperoxide, 0.833 mM for hydrogen peroxide and 0.375 mM for GSH.

Further studies on characterization of SGH–Px from pig platelets are in progress.

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Department of Biochemistry
Institute of Biochemistry
University of Lodz
Poland

Halina M. Żbikowska, Barbara Wachowicz

IZOLOWANIE I CZĘŚCIOWE OCZYSZCZANIE PEROKSYDAZY GLUTATIONOWEJ Z PŁYTEK KRWI ŚWINI

Metodą Maddipati i Marnett (1987) wyizolowano i częściowo scharakteryzowano peroksydazę glutationową z płytek krwi świni. Proces oczyszczania przebiegał kolejno poprzez wysalanie siarczanem amonu, hydrofobową chromatografię na złożu Phenyl–Sepharose CL–4B oraz chromatografię jonowymienną. Elektroforeza w żelu poliakryloamidowym z SDS (w obecności β-merkaptoetanolu) enzymu otrzymanego w ostatnim etapie oczyszczania ujawniła obecność podjednostki o masie cząsteczkowej 23 kDa. Wykazano, że w płytkach dominującą formą GSH–Px jest selenozależna peroksydaza glutationowa, która wykazuje ok. 86% całkowitej aktywności tego enzymu. Selenin sodowy powodował wzrost aktywności otrzymanego enzymu, podczas gdy jony miedzi, NEM i cisplatyna była inhibitorami peroksydazy glutationowej.