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Modulation of glyceraldehyde 3 phosphate dehydrogenase activity and tyr-phosphorylation of Band 3 in human erythrocytes treated with ferriprotoporphyrin IX

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ABSTRACT

Erythrocyte glyceraldehyde-3-phosphate dehydrogenase (G3PD), is a glycolytic enzyme normally inhibited upon binding to the anion transporter Band 3 and activated when free in the cytosol. We have previously reported that ferric protoporphyrin IX (FP) enhances G3PD activity in human erythrocytes (RBC). This could be due to two mechanisms considered in this work: Band 3 tyrosine phosphorylation or oxidative damage of specific G3PD binding sites in the membrane. In both cases binding of G3PD to the membrane would be prevented, leading to the enhancement of G3PD activity. Here, we show that FP induces a dose- and time-dependent phosphorylation of tyrosine 8 and 21 of Band 3, as confirmed by the recruitment of SHP2 phosphatase to the membrane. It appears that Band 3 phosphorylation is due to the oxidation of critical sulfydryl groups of a membrane phosphatase (PTP). Data on membrane localization, Mg^{2+} dependence, sensitivity to thiol oxidizing agents and protection by *N*-acetylcysteine (NAC) and DTT strongly suggest the involvement of PTP1B, the major PTP of human RBC associated to and acting on Band 3. However, FP activates G3PD even when Band 3 phosphorylation is inhibited, therefore phosphorylation is not the mechanism underlying G3PD activation by FP. The capacity of NAC of counteracting the stimulatory activity of FP, supports the hypothesis that FP might induce the oxidative damage of specific G3PD binding sites in the membrane, causing the displacement of the enzyme into the cytosol and/or the release from its binding site and therefore its activation.

1. Introduction

Haem (ferric protoporphyrin IX, FP) is a product of haemoglobin degradation which accumulates in the plasma of patients with malaria or haemolytic anaemia's [1] or inside the red blood cells (RBC) in haemoglobinopathies or G6PDH deficiency [2,3]. Through insertion into the lipid bilayers, FP causes potassium leak and cell swelling [4] and catalyses lipid and protein oxidation leading to destabilization of the cell membrane and RBC lysis [4–7].

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We have also reported that haem exerts opposite effects on glyceraldehyde-3-phosphate dehydrogenase (G3PD), a membrane bound glycolytic enzyme that is normally inhibited upon binding to the N-terminus of the anion transporter Band 3 and shifts to an highly active conformation when free in the cytosol [8]. We have shown that G3PD is partially inactivated by FP through oxidation of critical thiols [7]. Some of the activity is restored by dithiothreitol (DTT), but some critical thiols are irreversibly oxidated or modified by covalent crosslinking to products of FP-induced lipid peroxidation. In addition, following FP insertion into the membrane, some G3PD molecules are activated in a membrane-bound state or through displacement into the cytosol [7]. To clarify the mechanisms underlying FP-induced activation of G3PD, we have investigated whether FP is able of inducing tyrosine phosphorylation of Band 3 in intact RBC or isolated RBC membranes. In fact it has been shown that phosphorylation of the specific tyr-8 residue at the N-terminus of the cytoplasmic domain of Band 3, modulates glycolysis by preventing the binding of G3PD and of other glycolytic enzymes and leading to their activation [9–11]. Band 3 phosphorylation is stimulated by hypertonic NaCl, high Ca^{2+} or Mg^{2+} ions [12–14] or H_2O_2 [11] through the modification of the balance between the activity of protein tyrosine kinases (PTKs) $p72^{syk}$ and *Src* kinases [15,16] on the one hand, and phosphotyrosine phosphatases (PTPs) [17], on the other hand.

2. Materials and methods

2.1. Materials

Fresh human blood with CPD (citrate/phosphate/dextrose) as anticoagulant was obtained from healthy donors.

All biochemicals and anti-Band 3 monoclonal antibody (Ab) were purchased from Sigma; anti phosphotyrosine (P-Tyr) monoclonal Ab from Chemicon; staurosporine from Calbiochem; anti SHP-2 rabbit polyclonal Ab from Santa Cruz Biotechnology; anti mouse IgG and anti rabbit IgG peroxidase-linked, nitrocellulose membrane and the enhanced chemiluminescence developing system (ECL) from Amersham Bioscience.

A stock solution of 8 mM FP in 0.02 N NaOH was made daily and then diluted to the appropriate concentration in isotonic phosphate buffered saline (PBS). The final NaOH concentration in the test samples was negligible. The haem equivalents were quantified by dissolving an aliquot in 1 N NaOH and reading the absorbance at 385 nm (ε_{385} haematin = 6.1×10^4 M cm^{-1}).

2.2. Preparation of RBC and RBC membranes (ghosts)

Aliquots of blood were centrifuged at 1850 × *g* at 4 °C for 5 min, the buffy coat removed and the erythrocyte pellet washed three times with 10 volumes of cold (4 °C) PBS. Cells were gently resuspended with PBS-5 mM glucose and used immediately. Membranes were prepared by hypotonic lysis of control or FP exposed RBCs in 20 vol. of 5 mM $NaHPO_4$ buffer pH 8.0, containing 0.1 mM EDTA and 0.2 mM phenylmethylsulfonyl fluoride (PMSF), centrifugation at 25,000 × *g* (30 min, 4 °C) and extensive washing until free of haemoglobin. White ghosts were stored at −80 °C in a small volume of the same buffer containing a protease inhibitor cocktail (Sigma) (5 μl/200 μg proteins).

2.3. RBC and RBC ghost treatment

0.5–1 ml RBCs at 10% haematocrit (Htc) in PBS-5 mM glucose or white ghosts (400 μg protein/100 μl 5 mM $NaHPO_4$ buffer) were treated with different doses of FP. Incubation was performed for different times at 37 °C under shaking. In some experiments, the RBC suspensions or membranes (control or prepared from RBC pretreated with FP) were preincubated in the presence of inhibitors, antioxidants or other substances as described in Sections 3 and 4. At the end of the incubation, RBC or membranes were immediately processed and subjected to G3PD assay or SDS-PAGE and Western blotting analysis as described below. The state of oxidation of FP was evaluated by UV spectrometry. To this purpose ghosts treated with FP with or without NAC, as described in Fig. 3, were extracted in a mixture of 5 mM $NaHPO_4$ buffer/DMSO (60:40 by vol.) and the UV spectra recorded and compared to the spectra of standard ferric and ferrous protoporphyrin IX preparations dissolved in the same buffer/DMSO mixture. Ferrous protoporphyrin IX was prepared by reduction in the presence of 0.9% sodium dithionite and the spectrum recorded under nitrogen atmosphere. Ferric and ferrous protoporphyrin IX showed a maximum of absorbance at 400 and 430 nm, respectively.

2.4. G3PD activity

RBCs treated with FP were pelleted by centrifugation at 1850 × *g* for 10 min, washed twice with cold PBS (4 °C) to remove the unbound porphyrin and lysed with a proper volume of water just before the enzymatic assay. G3PD activity was monitored at 340 nm by the decrease in NADH in the coupled reaction described by Beutler [18] in a reaction mixture containing 82.5 mM triethanolamine buffer pH 7.6, 6 mM glycerate-3-P, 1.1 mM ATP, 0.9 mM EDTA, 1.7 mM $MgSO_4$, 0.2 mM NADH and 14.8 kU PGK. Enzyme activity was referred to the RBC content of haemoglobin calculated from the absorbance at 412 nm (A_{412}).

2.5. PTP activity

PTP was estimated by using *p*-nitrophenyl phosphate disodium salt (*p*-NPP) as substrate according to published procedures [19] with minor modifications. Membranes (50 μg protein) from control or FP treated RBC were incubated with 15 mM *p*-NPP at 37 °C for 30 min in 100 μl of buffer A (10 mM Tris buffer, pH 7.4 containing 20 mM Mg^{2+} and 50 nM okadaic acid to inhibit protein Ser/Thr phosphatases) and PTP activity measured in different experimental conditions. The reaction was stopped by 500 μl 0.1 M NaOH. Samples were centrifuged and the release of *p*-nitrophenol in the supernatant was estimated from the absorbance at 410 nm.

2.6. Gel electrophoresis and Western blot analysis

Immediately after treatment (to better preserve the phosphorylation state of the proteins) packed RBCs (500 μg haemoglobin) were diluted in 6 vol. of loading buffer under reducing

conditions (100 mM DTT) and boiled for 5 min [13]. Proteins were then separated by 10% SDS-PAGE [20]. When SDS PAGE was performed on the membranes of pretreated RBC, membranes were prepared in the presence of 1 mM Na_3VO_4 as phosphatase inhibitor and about 20 μg of proteins were solubilized in 2× loading buffer and incubated for 15 min at 60 °C. After SDS PAGE, proteins were transferred to nitrocellulose membrane at 100 mA, overnight. Blots were blocked with 10% non-fat dry milk in Tris buffered saline-1% Tween-20 (TBS-T) for anti-Band 3 and anti SHP-2 immunoblotting or 1% BSA in TBS-T for anti P-Tyr immunoblotting and incubated for 1 h at room temperature with the primary antibodies diluted in 5% no-fat milk in TBS-T. Blots were washed in TBS-T, followed by incubation at room temperature for 1 h with the appropriate secondary peroxidase conjugated Ab anti-mouse IgG or anti rabbit IgG in TBS-T, washed and finally detected with ECL. Blots were quantified by densitometric analysis (CAMAG VideoScan).

3. Results

3.1. Band 3 phosphorylation in intact RBC

Experiments were performed on RBC incubated at 37 °C with different doses of FP for 1 h, or with 40 μM FP over time (15–180 min). Aliquots of each sample containing 500 μg of Hb were submitted to SDS-PAGE immediately after the incubation. Staining with anti P-Tyr Ab showed that FP induces a dose and time-dependent increase in the tyrosine phosphorylation of Band 3 (Fig. 1a and b). As expected, incubation of RBC in the presence of hypertonic NaCl (900 m-osM) or H_2O_2 (1 mM) also resulted in significant Band 3 phosphorylation (Fig. 1a, lane 2 and 3) [11,13]. The densitometric measurement indicated a 5-fold increase of Band 3 phosphorylation after 2 h exposure of intact RBC to 40 μM FP (data not shown). These conditions were chosen for most of the subsequent experiments. The same dose- and time-dependent pattern of phosphorylation of Band 3 was observed in membranes isolated from RBC pre-treated with FP (data not shown), indicating that haemoglobin does not interfere with the results when SDS-PAGE is performed on the whole RBC, as reported by Minetti [21]. The identity of the phosphorylated Band 3 was shown using anti-Band 3 Ab, staining a protein with a molecular weight (95 kDa) comparable to that of the phosphorylated band (Fig. 1a and b).

3.2. FP-modulated activity of PTKs and PTP

FP-induced Band 3 phosphorylation was inhibited by 30 min treatment of RBC with 3 μM staurosporine, a known inhibitor

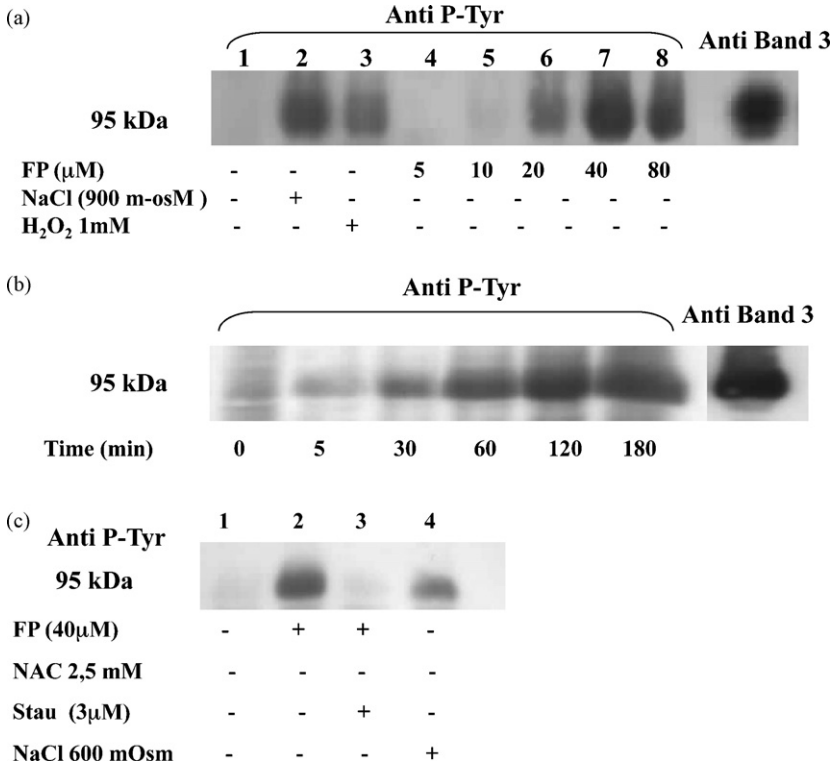

Fig. 1 – FP-dependent tyrosine phosphorylation of Band 3 in human erythrocytes. (a) RBC (10% final Htc) were incubated at 37 °C in PBS-5 mM glucose containing different amount of FP (60 min), NaCl 900 m-osM (15 min) or 1 mM H_2O_2 (15 min). (b) RBC (10% final Htc) were incubated at 37 °C in PBS-5 mM glucose containing 40 μM FP over time. (c) RBC (10% final Htc) were pre-incubated at 37 °C for 30 min in PBS-5 mM glucose in the absence (lane 2) or in the presence (lane 3) of 3 μM staurosporine and then treated with 40 μM FP for 2 h. Lane 1 control cells; lane 4, cells after 15 min in hyperosmotic conditions. After incubation, 500 μg Hb of the suspension were subjected to SDS PAGE (10%), transfer to nitrocellulose and immunodetection with anti-P tyr monoclonal Ab or anti-Band 3Ab and enhanced chemiluminescence. Anti-Band 3 in (b) comes from a gel run in parallel.

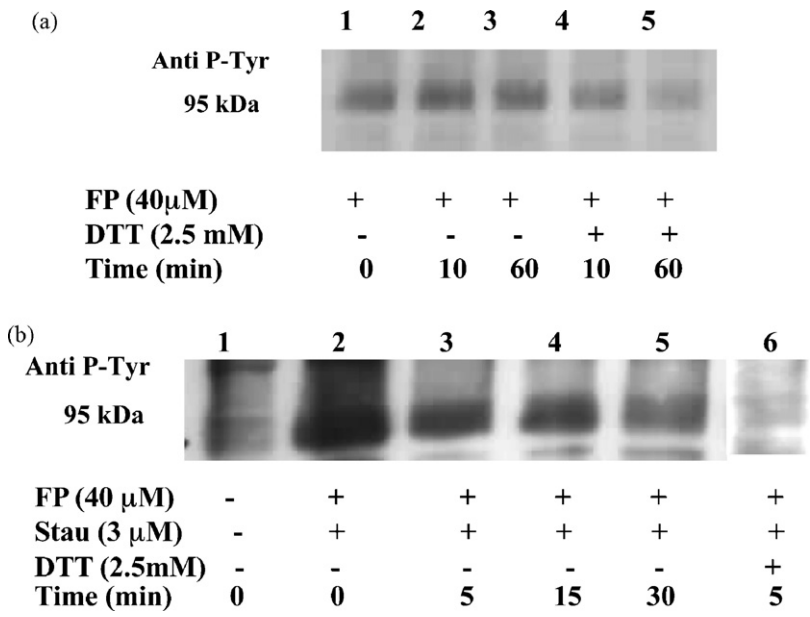

Fig. 2 – Activity of phosphatases in FP pre-phosphorylated intact RBC and effect of reducing agents. (a) RBC pre-phosphorylated with 40 μM FP (2 h, 37 °C) washed and reincubated over time in the absence (lane 1–3) or presence (lane 4–5) of 2.5 mM DTT. (b) RBC pre-phosphorylated with 40 μM FP (2 h, 37 °C) (lane 2) were added with 3 μM staurosporine and aliquots taken at different times (lanes 2–5). Lane 6, RBC added with staurosporine and 2.5 mM DTT. After incubation samples were processed as described in the legend of Fig. 1.

of the catalytic domain of $p72^{syk}$, a tyrosine kinase present in human erythrocytes [16] (Fig. 1c, lane 3). In RBC pretreated with 40 μM FP, Band 3 dephosphorylation did not occur spontaneously (Fig. 2a, lane 1-3), but was efficiently reversed, in a time-dependent manner, by the thiol reducing agent DTT (Fig. 2a, lane 4–5). When FP-treated RBC were incubated with staurosporine, a time-dependent decrease of Band 3 P-Tyr was observed even in the absence of DTT (Fig. 2b, lane 2–5). However, dephosphorylation was slow and still incomplete after 30 min indicating that the accumulation of P-Tyr is due to the inability of PTP to dephosphorylate Band 3. When DTT was added together with staurosporine the disappearance of P-Tyr was much faster and complete within 5 min of treatment (Fig. 2b, lane 6).

3.3. *Band 3 phosphorylation in RBC isolated membranes*

Ghosts prepared from control RBC were incubated in 5 mM $NaHPO_4$ buffer containing 5 μM ATP/10 mM Mg^{2+}, for 30 min at 37 °C with 40 μM FP in the presence or not of 2.5 mM NAC or DTT. A parallel incubation was performed in the presence of the phosphatase inhibitor sodium vanadate (0.05 or 0.1 mM) or 900 m-osM NaCl. As shown in Fig. 3a, Band 3 phosphorylation was not observed in membranes incubated with ATP/Mg^{2+} alone (lane 1) but was strongly stimulated by FP (lane 2) and vanadate [19] (lane 5 and 6) and prevented by the thiol reducing agents NAC or DTT (lane 3 and 4). A further proof of the inactivation of PTP is shown in Fig. 3b. Membranes prepared from RBC pre-phosphorylated by treatment with 40 μM FP have high levels of Band 3 phosphorylation (lane 1). Differently from intact RBC, when the pre-phosphorylated membranes were incubated in the presence of DTT, only a slight Band 3 dephosphorylation occurred (lane 2). The addition of Mg^{2+}, present in intact RBC, but absent in the purified membranes, was necessary to significantly reactivate (up to 90%) Band 3 dephosphorylation (lane 3).

PTP inactivation was also shown by the exogenous substrate *p*-NPP. As shown in Table 1, PTP activity on *p*-NPP was significantly lower in FP containing membranes compared to control. In addition, in agreement with Zipser et al. [19] and the experiments on Band 3 phosphorylation, PTP activity was completely inhibited by vanadate and in the absence of Mg^{2+}, partially inhibited by Mn^{2+} and enhanced by DTT, both in control and FP containing membranes. As expected, co-incubation of control membranes with FP reduced PTP activity by 80%.

Table 1 – PTP activity in RBC membranes (% of control)

Treatment	Untreated	FP- treated
Buffer A	100	$62 \pm 3^{*}$
Buffer A without Mg^{2+}	$6 \pm 4^{*}$	$5 \pm 2^{*}$
Buffer A + 1 mM vanadate	0	0
Buffer A + 20 mM Mn^{2+}	$27 \pm 2^{*}$	$25 \pm 3^{*}$
Buffer A + 40 μM FP	$22 \pm 2^{*}$	ND
Buffer A + 5 mM DTT	$133 \pm 10^{*}$	110 ± 6

Membranes were prepared from control RBC or RBC pretreated with 40 μM FP for 2 h at 37 °C. PTP activity was measured using *p*-NPP as substrate as described in Section 2 and the results expressed as percentage of the activity measured in control samples (untreated membranes in Buffer A). The results represent the mean ± S.D. of triplicate samples from a representative experiment repeated three times.

* $p < 0.001$ vs. control.

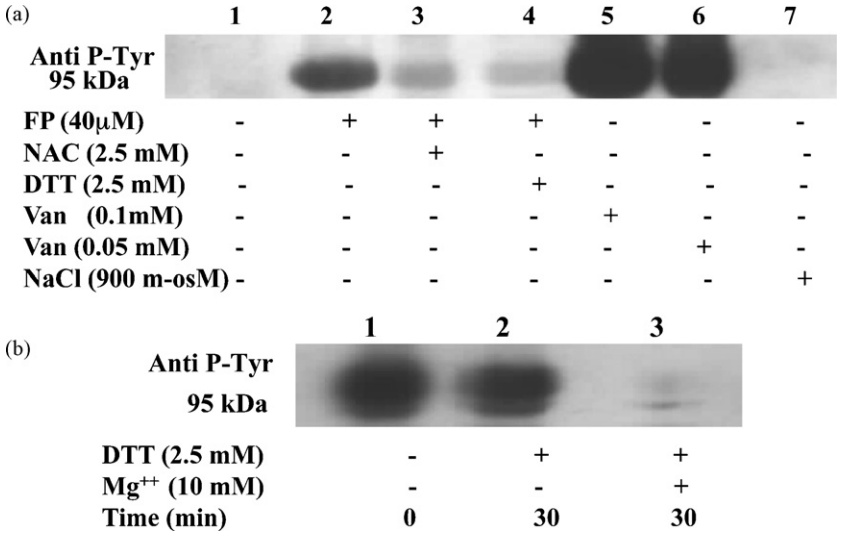

Fig. 3 – FP-dependent tyrosine phosphorylation of Band 3 and phosphatase activity in isolated membranes. (a) Ghosts from control RBC (200 μg protein) were resuspended in 50 μl of 5 mM Na phosphate buffer containing 5 μM ATP/10 mM Mg^{2+} and incubated for 30 min at 37 °C in the absence (lane 1) or presence of 40 μM FP (lane 2), 40 μM FP, 2.5 mM. NAC (lane 3), 40 μM FP, 2.5 mM DTT (lane 4), 0.1 mM or 0.05 mM vanadate (lanes 5 and 6) and 900 m-osM NaCl (lane 7). (b) Ghosts prepared from RBC pre-phosphorylated with 40 μM FP (2 h, 37 °C) (lane 1) were incubated for 30 min 37 °C in the presence of 2.5 mM DTT (lane 2) or 2.5 mM. DTT/10 mM Mg^2. After incubation 20 μg proteins were processed as described in the legend of Fig. 1.

We then investigated whether FP could elicit the recruitment to the membrane of the cytosolic SHP-2 tyrosine phosphatase. SDS/PAGE and immunoblotting with anti SHP-2 Ab show that following RBC treatment with FP, SHP-2 is recruited to the membrane (Fig. 4, lane 2). As expected, the same effect was observed in the presence of vanadate (lane 4) [22] and was abolished by the *Syk* inhibitor staurosporine (lane 3).

3.4. *FP induced modulation of G3PD activity*

FP induces a significant increase of the total G3PD activity (Table 2). As previously reported [7], most of the enzyme is activated by FP in the membrane bound state rather than displaced into the cytosol. Triton X-100, releasing the enzyme from Band 3 [23], causes a significant increase (about 2.8-fold) of G3PD activity only in control cells, demonstrating that in FP exposed RBC the enzyme activity is already present in an active state. It is possible that more G3PD remains in the

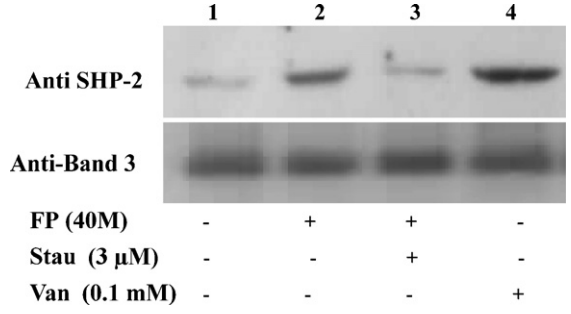

Fig. 4 – Recruitment of SHP2 to the membrane following FP treatment. Ghosts were prepared from RBC control (lane 1), pre-treated with 40 μM FP (2 h, 37 °C) in the absence (lane 2) or presence of 3 μM staurosporine (lane 3) or with 0.1 mM vanadate (lane 4) and subjected to SDS PAGE, immunodetection with anti SHP-2 Ab or anti Band 3 Ab and enhanced chemiluminescence.

Table 2 – Effect of FP on G3PD activity of human RBC in different experimental conditions

Treatment[a]	G3PD activity (% of controls)	G3PD activity (U/gr Hb)
Control (no additive)	100	26.3 ± 4.1
Control in the presence of detergent[b]		73.0 ± 6.7
40 μM FP	208 ± 36**	55.0 ± 9.0
40 μM FP in the presence of detergent[b]		58.2 ± 8.2#
40 μM FP after 30 min exposure to staurosporine	245 ± 20	
40 μM FP after 30 min exposure to 5 mM NAC	155 ± 41.6***	
1 mM H_2O_2	286 ± 41.3**	
1 mM H_2O_2 after 30 min exposure to 5 mM NAC	200 ± 21.0*	

Results are the mean ± S.D. of 3–5 determinations made in triplicate.
[a] Intact RBC (10% Htc) were exposed to 40 μM FP for 60 min and G3PD activity measured as described in Section 2.
[b] G3PD activity was determined in the RBC lysate after 2 min incubation with Triton X-100 (0.02% final concentration) to solubilize the membrane bound enzyme.
* $p < 0.005$ vs. 1 mM H_2O_2.
** $p < 0.001$ vs. control.
*** $p < 0.005$ vs. 40 μM FP.
# $p < 0.05$ vs. control in the presence of Triton X-100.

soluble, more active form, due to the FP-induced oxidative damage of some binding sites on the membrane. The lower activity obtained in the presence of Triton in FP treated cells, compared to control, might be due to the oxidative inactivation of G3PD molecules, as reported [7].

To clarify whether the FP-induced activation of G3PD is dependent upon the phosphorylation of Band 3, RBC were incubated for 30 min with staurosporine before the exposure to 40 μM FP (60 min, 37 °C). Staurosporine did not inhibit the enhancement of G3PD activity caused by FP. Similarly, 1 mM H_2O_2, a Band 3 phosphorylating agent (Fig. 1a), significantly stimulated G3PD and the enzyme activity was not affected by staurosporine (not shown). 5 mM NAC significantly reduced G3PD activation induced by both H_2O_2 and FP supporting the hypothesis that G3PD activity might be modulated through the oxidation of critical binding sites in the membrane.

4. Discussion

Tyrosine phosphorylation of erythrocyte Band 3 was shown in intact RBC as well in membranes isolated from RBC pretreated with FP. The inhibiting activity of staurosporine, suggests the involvement of $p72^{syk}$, a tyrosine kinase present in human erythrocytes [16]. Piceatannol, the most commonly employed inhibitor of $p72^{syk}$, could not be used in these experiments because of its strong oxidant activity towards haemoglobin. A further evidence supporting the activity of $p72^{syk}$ comes from the phosphorylation experiments performed in isolated membranes. In fact, the kinase responsible of Band 3 phosphorylation is a membrane bound enzyme, that remains stably associated to the membrane in the conditions used for RBC lysis and membrane preparation. Its activation, differently from the hypertonic medium (Fig. 3, lane 7), does not require cytosolic effectors or an intact cell structure, therefore excluding the involvement of *Src* $p56/p53^{lyn}$ kinases, whose association with the membrane is unstable in the conditions of lysis [16]. The FP-induced recruitment to the membrane of the cytosolic SHP2 phosphatase (Fig. 4) strongly supports the phosphorylating activity of $p72^{syk}$ on tyrosine 8 and 21 of Band 3. In fact, accordingly to Bordin et al. [22], a primary phosphorylation of Band 3 at Tyr 8 and 21 by *Syk* is required for the activation of the *Src* kinase which catalyzes the secondary phosphorylation at Tyr 359, the specific docking site of SHP2 phosphatase. The sequential action of *Syk* and *Src* kinases is further confirmed by the observation that the *Syk* inhibitor staurosporine abolishes the association of SHP-2 with the membrane (Fig. 4, lane 3).

In isolated membranes, as well in intact RBC, Band 3 phosphorylation can be prevented or reversed under reducing conditions (NAC or DTT) suggesting that PTP inability to dephosphorylate Band 3 is involved in the accumulation of P-Tyr and that the FP-induced loss of activity of membrane associated PTP, whose activity usually outweighs that of PTKs [24], might be due to oxidative damage of critical sulfydryl groups. The formation of mixed disulfide (possibly glutathionylation or PTP-Band 3 mixed disulfides) might account for the PTP inhibition [17,25].

Finally, the PTP features (membrane localization, sensitivity to thiol oxidizing agents such as FP [6] and Mg^{2+} dependence) strongly suggest that PTP is related to PTP1B, the major PTP of human RBC associated to and acting on Band 3 [17,19]. In conclusion, Band 3 tyrosine phosphorylation induced by FP is dependent upon the oxidative inactivation of PT1B phosphatase rather than the activation of PTKs.

Analysis of the UV spectra of FP pre-incubated with RBC membranes, with or without NAC, were identical to that of ferric protoporphyrin IX (data not shown) suggesting that in our experimental conditions the large majority of FP is in the iron III state of oxidation. In agreement, we did not find any other peak when the solution was flushed with carbon monoxide which is known to complex iron II haem with a maximum of absorbance at 419 nm. We can also exclude that the oxidative effects exerted by FP might be due to the generation of ferryl species [Fe(IV) = O]. Oxoferryl complexes have been shown to occur in different haem-proteins [26]. In our experimental conditions a proteic environment is lacking, therefore production of iron–oxygen complexes would lead to autooxidation with degradation and bleaching of the porphyrin itself. This does not seem the case in our experiments since no decrease in the maximum value of absorbance (400 nm) was observed in FP preincubated with the membranes (data not shown).

Tyr phosphorylation of Band 3 has been reported to prevent the binding of G3PD and lead to its activation [10,11]. However, as shown in Table 2, FP activates G3PD even when Band 3 phosphorylation is inhibited by staurosporine. This result demonstrates that in the presence of FP (and H_2O_2, as well), the phosphorylation of Band 3, though present, is not the mechanism responsible of activation of G3PD. This is also supported by the different kinetics of FP-induced Band 3 phosphorylation and G3PD activation, reaching a plateau after 2 h or 30 min of incubation, respectively (Fig. 1b and ref [7]). The stimulation of G3PD by H_2O_2 and the capability of NAC of counteracting the stimulatory activity of FP, raise the possibility that FP might activate G3PD through the oxidative damage of critical binding sites in the membrane preventing the association of enzyme and therefore maintaining more enzyme in the soluble/more active form. Further experiments are in progress to clarify this issue.

Band 3 phosphorylation has been found in sickle and β thalassemic red cells [27,28] and showed to increase membrane rigidity [29] and promote echinocyte transformation [30]. Impaired RBC deformability may contribute to the microcirculatory obstruction and accelerated removal of RBC seen in malaria or haemoglobinopathies. In light of our previous and present data, we can postulate that FP accumulating in the plasma of patients or inside RBC in malaria and other haemolytic anaemias, might contribute to the alteration of the rheological properties of RBC. Impaired deformability of RBC has been shown in vitro after exposure to FP [31] as well as in uninfected RBC from malaria patients [32]. Our finding on the capability of NAC of preventing FP-induced Band 3 phosphorylation is in agreement with the observation that NAC can both prevent and reverse the FP-induced haemolysis and decrease in red cell deformability [6,31]. These beneficial effects strongly support the use of NAC in clinical practice as adjunctive treatment in severe malaria anaemia and other RBC dysfunctions [33].

Acknowledgements

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