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To the editor:

Beta 2 glycoprotein I is a substrate of thiol oxidoreductases

Beta 2 glycoprotein I (β2GPI) is an abundant plasma protein recognized as the major autoantigen in the antiphospholipid syndrome. Although the crystal structure of β2GPI has been resolved,[1,2] its normal function remains unknown. We have been intrigued by the presence of a C-terminal cysteine (Cys326), which forms a loop-back disulfide link in the fifth domain of β2GPI. In the current study we examined β2GPI's potential to participate in thiol exchange reactions with the thiol oxidoreductases thioredoxin-1 (TRX-1) and protein disulfide isomerase (PDI).

The incorporation of free thiols into β2GPI after reaction with TRX-1 or PDI was shown by labeling the products of this reaction with the selective sulfhydryl probe N^a-(3-maleimidylpropionyl) biocytin (MPB). The biotinylated proteins were visualized by Western blotting with streptavidin–horseradish peroxidase. Because β2GPI does not contain unpaired cysteines, no labeling was observed after incubation with MPB (Figure 1A). Free thiols could not be introduced into β2GPI by incubation with the reducing agent dithiothreitol (DTT) alone. However, free thiols could be introduced into β2GPI after incubation with the reduced forms of the thiol oxidoreductases TRX-1 and PDI, identifying β2GPI as a thiol oxidoreductase substrate (Figure 1A-C). An interesting effect caused by the reduction of β2GPI by TRX-1 was a marked decrease in the affinity of anti-β2GPI monoclonal and polyclonal antibodies as noted on the immunoblots.

To determine the cysteine residue(s) in the β2GPI molecule involved in thiol exchange reactions, β2GPI treated with the TRX-1/TRX-1 reductase/NADPH system and labeled with MPB was resolved on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE; Figure 1B). Gel bands were excised, digested, and analyzed by liquid chromatography–tandem mass spectrometry. Mass spectral data were searched using Mascot (Version 2.2; Matrix Science) or converted to MzXML file format using ReAdW (Version 4.0.2)[3] and submitted to the database search program X!Tandem (Release 2008.12.01).[4] The analysis revealed Cys326 to be predominantly labeled with biotin (F.H.P., S.R., M.Q., M.J.R., J.W.H.W., K.T., Y.I., J.Y.Z., R.G., J.C.Q., B.G., W.E.H., P.J.H., S.A.K., manuscript submitted). The structural features of the disulfide bond containing Cys326 and all disulfide bonds of the 2 structures of β2GPI (PDB 1C1Z and 1QUB) were determined using the disulfide bond analysis tool available at www.cancerresearch.unsw.edu.au/CRCWeb.nsf/page/Disulfide+Bond+Analysis.[5] The analysis showed that the Cys288-Cys326 disulfide is a −/+ right-handed hook (−/+RHHook) configuration in both crystal structures of the protein.[1,2] Although there is no other structural similarity with β2GPI, the active site disulfides of oxidoreductases like TRX-1 or PDI are +/− RHHooks. Of the 22 Cys residues in β2GPI, Cys326 stands out as being exposed to solvent. The solvent accessibility values for Cys326 are 117 (PDB ID 1C1Z) and 103 (PDB ID 1QUB) $Å^2$ for the 2 structures. This high solvent exposure is consistent with reduction of the Cys288-Cys326 disulfide bond by thiol oxidoreductases.

Thiol oxidoreductases are becoming increasingly recognized as important mediators of platelet function.[6] The prototype member, PDI, has been implicated in the activation of the fibrinogen receptor αIIbβ3[7] and tissue factor.[8] Several novel members of the thiol isomerase family have been recently shown to translocate to the platelet surface after platelet activation.[9] In the current issue of *Blood*, Ioannou et al have developed a sensitive and specific streptavidin-capture enzyme-linked immunosorbent assay (ELISA) to detect reduced β2GPI in plasma.[10] With the same methodology β2GPI is shown to be reduced after incubation with platelets, which can be attributed partially to the TRX-1 system (Figure 1D).

This study is the first to show the potential of β2GPI to participate in thiol exchange reactions. Our finding suggests that β2GPI may participate in redox processes in vascular biology.

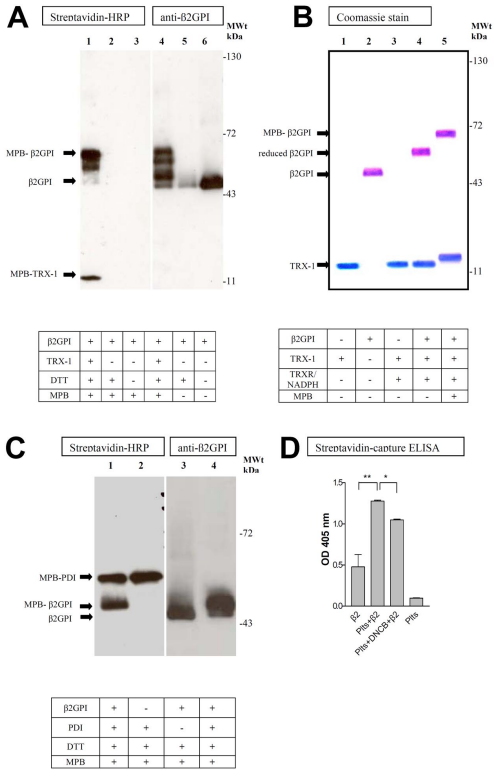

Figure 1. Free thiols introduced into β2GPI after reaction with TRX-1 and PDI; reduction of β2GPI on the platelet surface. Native or recombinant β2GPI was incubated with TRX-1 previously reduced by DTT (A) or TRX-R/NADPH (B) or with PDI reduced by DTT (C). Reduced TRX-1 was prepared by incubating 5μM TRX-1 with 25μM DTT or 10nM TRX-R/200μM NADPH for 1 hour at 37°C. Reduced PDI was prepared by incubating 500nM PDI with 50μM DTT. β2GPI (0.2μM) was incubated with the TRX-1 or PDI mixtures for 1 hour at 37°C. To label free thiols, N^a-(3-maleimidylpropionyl) biocytin (MPB; 100μM) was added to the β2GPI/TRX-1 or PDI solutions for 10 minutes at 37°C. The reaction was quenched with glutathione (200μM) for 10 minutes at 37°C. All reactions were performed in 20mM HEPES, 0.14M NaCl buffer, pH 7.4. The reactions were resolved under nonreducing conditions on SDS-PAGE (4%-12%) and then transferred to polyvinylidene fluoride membranes. MPB-labeled β2GPI was probed with streptavidin–horseradish peroxidase (1:1000). β2GPI was detected with the 4B2E7 monoclonal antibody 3.5 mg/mL or rabbit anti-β2GPI Ab 1.4 mg/mL. (A) Incorporation of MPB into β2GPI (at ~ 70 kDa) after treatment with TRX-1 (lane 1). MPB cannot be introduced into β2GPI after treatment with the reductant DTT alone defining β2GPI as a substrate of TRX-1 (lane 2). Lanes 4 and 5 show detection of the reduced β2GPI forms on SDS-PAGE and the decreased immunoreactivity of anti-β2GPI monoclonal antibody to reduced β2GPI (± MPB) compared with nonreduced β2GPI (lane 6). (B) Coomassie staining of β2GPI incubated with TRX-1/TRX-R/NADPH/MPB and separated on SDS-PAGE shows one β2GPI band at ~ 70 kDa in accordance with the MPB-labeled β2GPI band in the streptavidin–horseradish peroxidase blot (panel A lane 1) and the first β2GPI immunoreactive band on the anti-β2GPI blot (panel A lane 4). The remaining 3 β2GPI immunoreactive bands (between 50 and 70 kDa) apparent in the anti-b2GPI blot (panel A lane 4) are not detected on the Coomassie, showing that these β2GPI products have very low concentrations. (C) Incorporation of MPB into β2GPI after reaction with PDI. MPB-labeled β2GPI, after reduction with PDI, showed a minor shift in molecular size on the SDS PAGE (~ 50 kDa, panel C lane 1) in comparison to TRX-1 treated β2GPI (panel A lane 1), showing that TRX-1 had a greater effect than PDI on the biotin labeling or denaturing of β2GPI. Numbers indicate the molecular weight markers. (D) Detection of reduced β2GPI on the platelet surface by a β2GPI-specific streptavidin-capture ELISA. Platelets (in buffer or pretreated with the TRX-R inhibitor 1-chloro-2, 4-dinitrobenzene [DNCB]) were incubated with or without β2GPI. MPB was added to label any free thiols formed. After acetone precipitation to remove nonincorporated MPB, the precipitated MPB-labeled proteins were applied to a streptavidin plate, which captured MPB-labeled proteins including (reduced) β2GPI. β2GPI in 20mM HEPES, 0.14M NaCl buffer, pH 7.4 buffer alone plus MPB served as a negative control (first column). As a positive control β2GPI reduced by TRX-1/TRX-/NADPH plus MPB was used. β2GPI incorporated MPB after incubation with platelets which was partially inhibited by DNCB. Platelets alone plus MPB gave a negligible signal on this ELISA. OD indicates optical density; β2, β2GPI; M, MPB; and TRN, TRX-1/TRX-R/NADPH. Data are expressed as mean ± SD, n = 3 in triplicate. $^*P < .02$; $^{**}P < .01$.

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