**β₂GPI expression and identification**

_Molecular cloning of β₂ glycoprotein I (β₂GPI) cDNA._ Total RNA was extracted from HeLa cells using Trizol reagent (Life Technologies, Carlsbad, CA). Primers specific for β₂GPI (5’-GGACGGACCTGTCCCAAGC-3’ and 5’-TTAGCATGCTTTACATCGGA-3’) were synthesized. The cDNA encoding the mature human β₂GPI was obtained by performing reverse transcription-polymerase chain reaction (RT-PCR) and cloned into pCR2.1 vector (Life Technologies, Carlsbad, CA) to construct pCRhuβ₂GPI. Using pCRhuβ₂GPI as a template, primers 5’-TATCGAATTCGGACGGACCTGTCCCAAGC-3’ and 5’-AATCGCGGCCGCTTAGCATGCTTTACATCGGA-3’ were used for the next round of PCR. The resultant β₂GPI cDNA fragment with EcoR I and Not I restriction sites (underlined in primers) was cloned into pET28 vector (Novagen, Merck KGaA, Darmstadt, Germany) to construct pEThuβ₂GPI.

_Recombinant β₂GPI (rβ₂GPI) expression._ *Escherichia coli* strain BL21(DE3) carrying pEThuβ₂GPI was grown and harvested after optimal induction of recombinant protein expression. Cells were lysed with a French Pressure cell press (Thermo IEC, Needham Height, MA) and rβ₂GPI protein was purified by metal chelation chromatography as described previously for the purification of histidine-tagged recombinant protein (Lin et al., 2006). The purified rβ₂GPI protein was resolved by SDS-PAGE and analyzed by Western blot (Fig. i).

**Fig. i.** SDS-PAGE and Western blot analysis of the purified recombinant human β₂GPI (rβ₂GPI) protein. Recombinant protein was purified by metal chelation chromatography, resolved by 10 % SDS-PAGE (panel A, lane 1), and analyzed by Western blot (panel B, lane 1) using anti-His-tag antibody. M is prestained protein marker.
**Identification of rβ2GPI protein.** Purified rβ2GPI protein was analyzed by SDS-PAGE. Gel plugs containing rβ2GPI protein were collected and in-gel trypsin digestion of the protein was performed. The mass spectra were obtained by Ultraflex matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometer in reflectron mode and analyzed by using FlexAnalysis software (Bruker Daltonics, Bremen, Germany). Peptide masses obtained were searched against a comprehensive nonredundant protein sequence database (NCBInr) using the Mascot search (Perkins et al., 1999) for protein identification with the following parameters: trypsin digestion with a maximum of one missed cleavage, peptide with fixed carbamidomethylation at Cys and variable oxidation at Met, 50 ppm of peptide mass tolerance. The identity of the purified rβ2GPI protein was verified by MALDI-TOF mass spectrometry (Fig. ii).

**Fig. ii.** Identification of recombinant human β2 glycoprotein 1 (rβ2GPI) by mass spectrometry. Purified rβ2GPI was treated with trypsin, and the resultant peptides were analyzed by MALDI-TOF mass spectrometry. Mass spectrum (m/z) of peptides over 1000 daltons is shown (panel A). Peptide mass of human β2 glycoprotein 1 and its amino acid position (in parenthesis) are indicated. Matched peptides are underlined (panel B).
References