BIOCHEMICAL PHARMACOLOGY 72 (2006) 551–557

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Identification and characterization of triosephosphate isomerase that specifically interacts with the integrin αIIb cytoplasmic domain

Qiang-Yuan Liu [1], Martha Corjay [2], Giora Z. Feuerstein [1], Ponnal Nambi [1,*]

Du Pont Pharmaceuticals, Route 141 & Henry Road, Wilmington, DE 19880, USA

ARTICLE INFO

Article history:
Received 27 March 2006
Accepted 24 May 2006

Keywords:
Integrins
Triosephosphase isomerase
Platelet
Adhesion molecules GPIIb

Abbreviations:
TPI, triose phosphate isomerase
GST, glutathione S-transferase
ELISA, enzyme-linked immunosorbent assay

ABSTRACT

Integrin αIIb/β3 (IIb/IIIa), a platelet fibrinogen receptor, has been shown to play a critical role in thrombosis and hemostasis. However, the mechanisms by which ligands interact with the αIIb/β3 receptor is not very clear at this time. The interaction between the ligand, the receptor and the transmission of extracellular signals may involve the cytoplasmic domains of these integrins. The objective of this investigation was to identify novel proteins that interact with the cytoplasmic tail of αIIb. Using αIIb cytoplasmic tail as the bait and a yeast two-hybrid system, we have identified three separate clones containing inserts that encoded the same protein with different truncated N-terminals. Sequence analysis showed that the inserts of the three clones encoded a previously identified enzyme: triose phosphate isomerase (TPI). In addition, we demonstrated that TPI failed to interact with the integrin α2 tail, β3 tail and lamin, but showed a weak binding to the αV tail which shares the highest homology with αIIb tail among the integrin α family. Site-directed mutagenesis studies around the homology region indicated that the critical peptide sequence necessary for the interaction between TPI and αIIb tail is GFFKRNRPPLEE. Using RT-PCR, we have demonstrated the presence of TPI mRNA in platelets. In addition, experiments were also performed to demonstrate specific binding of TPI to αIIb using an ELISA and fusion protein. Taken together, these data suggest that TPI specifically interacts with αIIb and may play a critical role in αIIb/β3-mediated platelet function.

1. Introduction

Integrin-mediated adherence of cells to extracellular matrices is important for a variety of physiological processes such as hemostasis, angiogenesis, and cell differentiation. Integrins are noncovalent α/β heterodimers. Each subunit contains a large extracellular region, a transmembrane domain, and a short cytoplasmic tail [1]. Integrins promote not only adhesion to components present within the extracellular matrix or on the surface of opposite cells but also transfer information in and out of a cell [1]. The adhesive functions of integrins can be regulated by intracellular processes referred to as "inside-out" signaling. Conversely, ligand binding to the extracellular domain of integrins initiates a cascade of intracellular events termed "outside-in" signaling that generates a large spectrum of cellular responses, such as cell migration, proliferation,

* *Corresponding author at*: Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA. Tel.: +1 484 865 8125.
E-mail address: nambip@wyeth.com (P. Nambi).
[1] Current address: Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA.
[2] Current address: Bluegrass College, 470 Cooper Drive, Lexington, KY 40506, USA.
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2006.05.020

differentiation, and gene expression [2]. Integrin cytoplasmic tails appear to be key elements in these bidirectional signaling pathways, despite their short size as compared with other signaling receptors and the absence of any demonstrable catalytic activity [3,4]. Integrin α and β cytoplasmic tails are thought to mediate signaling events through modifications of their own structural and spatial organization and/or through interactions with specific cytoplasmic components. Various proteins that bind to the cytoplasmic domain of IIb and IIIa have been identified and are likely to play a role in regulating integrin signaling functions. Some of these proteins, including cytoskeletal components such as talin, α-actinin, and focal adhesion kinase, have been shown to bind directly to the cytoplasmic tail of β1 or β3 in *in vitro* assays [5–9]. Other integrin-binding proteins have been identified using integrin cytoplasmic domains in a yeast two-hybrid system. For example, integrin cytoplasmic domain-associated protein [10] and integrin-linked kinase [11] have been identified using the β1-integrin cytoplasmic tail; endonexin and M-band protein skelemin were identified using the β3-integrin cytoplasmic domain [12,13]; and SEC7 homologue cytohesin was identified using the β2-integrin cytoplasmic domain [14]. CIB (calcium and integrin-binding protein) [15,16] and calreticulin [17,18] have been identified using IIb tail as bait.

The mechanism of integrin-mediated signaling and localization is unclear but is believed to involve the interaction of integrin cytoplasmic domains with specific proteins. In this study, we have identified the triose phosphate isomerase (TPI) that specifically interacts with IIb cytoplasmic domain by using a yeast two-hybrid system. Site-directed mutagenesis data suggested that the peptide sequence GFFKRNRPPLEE of the IIb tail is critical for the interaction of the TPI with the IIb cytoplasmic domain. *In vitro* binding studies indicated that TPI can bind to IIb using ELISA analysis and GST fusion protein.

2. Experimental procedures

2.1. Construction of the IIb tail bait

The DNA fragment encoding the cytoplasmic domain of human integrin IIb, integrin α2, integrin αV, integrin IIIa and lamin were amplified using RT-PCR (reverse transcriptase-polymerase chain reaction) from poly-A mRNA derived from platelets. The primer sequences were, IIb: forward 5′-GATGTGGAAGGTCGGCTTCTT, reverse 5′-CTCCCCCTCTTCATCATCT; α2: forward 5′-AGCTCTGGTTGCAATTTATGG, reverse 5′-GCTACTGAGCTCTGTGGT; αV: forward 5′-TATTTGTAATGTACAGGATGG, reverse 5′-AGTTTCTGAGTTTCCTTCACC; IIIa: forward 5′ –CTAAATTTGAGGAAGAACGC, reverse 5′ –GTGCCCCGGTACGTGATATTG; lamin: forward 5′-AGCGGCTCAGGAGCCCAGGT, reverse 5′-CATGATGCTGCAGTTCTGGG. The fragments were then subcloned into pHybLex/Zeo, a yeast two-hybrid bait vector (Invitrogen, CA), in frame with the LexA coding region, and the *E. coli* transformants were selected on low salt LB agar (10 g Tryptone, 5 g NaCL, and 5 g yeast extract in 1 L) plates containing 25 μg/ml Zeocin. DNA sequencing of bait plasmid indicated an in-frame fusion of the inserts to the LexA DNA binding domain.

2.2. Transformation of bait plasmid into yeast and bait behavior test

The two-hybrid bait plasmid was transformed into yeast stain EGY48/pSH18-34 (Invitrogen) using the lithium acetate method [19]. Transformants were selected on Ura-drop-out medium (Sigma, St. Louis, MO) plates containing 200 μg/ml Zeocin. The plates were incubated at 30 °C for 3 days and analyzed for the leucine prototrophy and β-galactosidase activity.

2.3. Yeast two-hybrid screening

A yeast two-hybrid system was used according to the supplied protocol (Invitrogen). The IIb tail bait was used to screen a human adult brain cDNA library fused to the V5 epitope-NLS-B42 activation domain (AD) of pYESTrp vector purchased from Invitrogen. The bait plasmid and the amplified library DNA were sequentially transformed into yeast strain EGY48/pSH18-34 (Invitrogen) using the lithium acetate method [19]. The transformants were selected at 30 °C on tryptophan-, leucine-, and uracil-drop-out medium plates containing 200 μg/ml Zeocin. After 6–10 days, Leu+ colonies were tested for β-galactosidase activity. The cDNAs of the Leu+ and LacZ+ double positive colonies were amplified by PCR using pYESTrp vector forward and reverse primers and the cDNA fragments were purified and sequenced.

2.4. RNA isolation and RT-PCR

Poly A^+ mRNA was isolated from platelets using a QIAGEN Kit (QIAGEN). The first-strand cDNA was synthesized from 0.1 μg of mRNA in a 20 μl reaction mixture containing 4 μl of 5× RT reaction buffer, 10 units of RNasin, 200 μM dNTP, 20 pM random primer, 20 units of reverse transcriptase. The mixture was incubated at 42 °C for 1 h and then at 53 °C for 30 min. The unhybridized RNA was then digested with 10 units of RNase H at 37 °C for 10 min. A 1 μl of the RT products was subjected to PCR amplification using TPI-specific primers. The primer sequences were TPI upstream: 5′-ATGGCGCCCTCCAGGAAGTTC; downstream: 5′-TCATTGTTTGGCATTGATGATG. Thirty-five cycles of amplification were performed in a themocycler at 94 °C (30 s), 57 °C (30 s), and 72 °C (1 min). The RT-PCR products were analyzed on a 1.2% agarose gel. The same amount of mRNA was used as a template in the PCR to verify that the band was amplified from cDNA.

2.5. Site-directed mutagenesis

Mutagenesis was employed according to the supplied protocol. Briefly, all primers were 5′ phosphorylated by T4 kinase. The selection and one of the mutagenic primers were simultaneously annealed to the target plasmid DNA as follows: 0.25 pmol of the plasmid, 25 pmol of the selection primer and 25 pmol of the mutagenic primer. The primers were extended with T7 DNA polymerase and the new strands were ligated with T4 DNA ligase. After digesting with the appropriate restriction enzyme, the newly generated mutant plasmids were transformed into XlmutS Competent Cells (Stratagene, La Jolla, CA), and grown in a liquid media

overnight. The plasmid DNA was isolated and again digested with the same restriction enzyme prior to being transformed into XL1-Blue competent cells. The Plasmid DNA was isolated from positive transformants and the inserts of interest were sequenced prior to further experimentation.

2.6. *In vitro binding studies using ELISA*

ELISA assay system was constructed according to the supplied protocol of ELISA Construction System (ZeptoMetrix Corporation, Buffalo, NY). Briefly, microtiter wells were coated with 5 μg/ml of purified TPI derived from rabbit muscle (Boehringer Mannheim). A 10 μg/ml of BSA was coated for a negative control. After blocking with ZeptoBlock (ZeptoMetrix Co.) and coating with ZeptoCoat buffer (Zeptometrix Co.), 20 ng/ml purified IIb/IIIa (purified using published procedure by Biotechnology group at Dupont) were added to the corresponding wells and incubated at 4 °C overnight. Wells were washed three times with PBST and incubated with IIb/IIIa complex-specific mAb (Accurate Chemical and Scientific Co., Westbury, NY) at RT for 1 h. After washing three times with PBST, the wells were incubated with anti-mouse secondary antibody conjugated with HRP for 1 h at RT. After the final wash with PBST, the amount of bound IIb/IIIa was determined using OD absorption.

2.7. *In vitro binding assays using recombinant proteins*

Glutathione S-transferase (GST) fusion constructs with TPI were made by subcloning the cDNA of the entire coding region of TPI into the expression vector pGEX-4T-3 (Amersham Pharmacia Biotech). The fusion proteins were expressed in *E. coli* strain BL12/pLysS(DE3) (Amersham Pharmacia Biotech). Protein expression was induced by the addition of isopropyl-β-D-thiogalactoside. Intact bacterial cells containing the expressed fusion proteins were collected by centrifugation at 10,000 × *g* for 10 min. Protein extracts were obtained by freezing and thawing the cell pellet and resuspending in GST purification buffer 1× PBS, 50 mM Tris–HCl, pH 8.0, 0.5 mM $MgCl_2$, 0.1% (v/v) Triton X-100, 1 mM phenylmethylsulfonyl fluoride plus 5 mM dithiothreitol. The protein extracts were immobilized onto glutathione Sepharose 4B by mixing in a 1.5 ml microcentrifuge tube for 2 h at 4 °C. The beads were washed three times with PBS to remove unbound proteins. The purified IIb/IIIa complex was allowed to interact with GST-fusion protein-coated glutathione Sepharose beads for 2 h at 4 °C. Any unbound proteins were removed through extensive washings. The proteins that remained bound to the immobilized GST beads were released by boiling in SDS gel sample buffer and analyzed by 10% SDS-polyacrylamide gel electrophoresis (PAGE), and immunoblotting with anti-IIb/IIIa antibody. Antibody binding was detected with peroxidase-conjugated secondary antibody (Amersham). After washing, ECL was performed using the procedure recommended by the manufacturer (Amersham).

3. Results

3.1. *Identification of αIIb cytoplasmic domain-interacting protein*

The entire cytoplasic domain of the IIb subunit (residues 1019–1039) was fused to the LexA DNA-binding domain and used as a bait to screen a human brain cDNA library. Of ~3 million independent transformants screened, about 40 grew in leucine minus medium and in secondary screen only 15 appeared to be galactose-dependent. Three of these colonies (colony #20, #29 and #59) also showed very strong galactosidase activity (blue color appeared within 20 min). The other Leu+ colonies either did not support galactosidase activity in the subsequent screening or had an insert that did not match

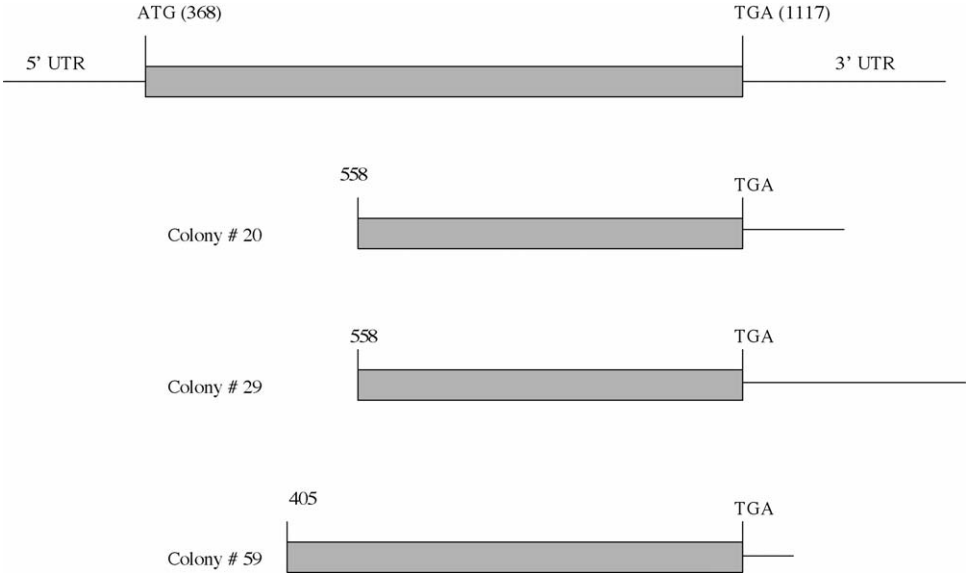

Fig. 1 – A diagram of triose phosphate isomerase mRNA and the insert sequences of the three double positive colonies. The boxed area represents the coding region of TPI. The numbers indicate the position of the nucleic acids. Start and stop codons of TPI mRMA are also indicated.

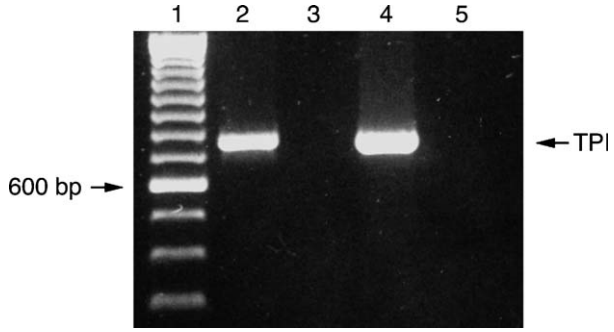

Fig. 2 – Expression of TPI in platelets. Lane 1: 100 base pair DNA ladder. Lane 2: TPI messenger was amplified from platelets mRNA by using RT-PCR analysis. Lane 3: same amount of platelet mRNA was used as PCR template (without RT step). Lane 4: TPI messenger in human heart mRNA. Lane 5: no fragment was amplified from human heart mRNA in a RT(−) reaction.

the coding region of known proteins or had short reading frames. The cDNA sequences of the three double positive colonies were purified for sequencing and shown to encode the same protein, but were truncated in different positions within the N-terminals (Fig. 1). After comparing sequences of the cDNA with known sequences in GenBank Database, we found that the inserts of the three positive colonies encoded a previously identified enzyme—triose phosphate isomerase (TPI).

3.2. *Expression of TPI in human platelets*

To determine whether TPI is expressed in human platelets, we isolated poly A mRNA from platelets and performed RT-PCR to amplify the entire TPI coding region (747 base pair in length). As shown in Fig. 2, a band at about 750 base pairs was amplified from platelet mRNA (Fig. 2, lane 2). The identity of this fragment was verified by DNA sequencing. To check for artifacts based on possible contamination of RNA by genomic DNA during mRNA isolation and RT-PCR, a reaction was performed using the same amount of mRNA as a template under identical conditions, but with no RT step (i.e., no added reverse transcriptase). No fragment was amplified from this RT(−) reaction (Fig. 2, lane 3). Human heart mRNA was used as a positive control for the RT-PCR reaction (Fig. 2, lane 4).

3.3. *Specificity of interaction between TPI and baits*

To determine whether TPI interacts with other baits, we segregated the original IIb bait plasmid from colony #59 and the pYESTrp-TPI plasmid was then cotransformed into the original EGY48/pSH18-34 yeast strain with the following baits: IIb tail, integrin α2 tail, integrin IIIa tail, Lamin, and null pHybLex/Zeo plasmid, respectively. The data demonstrated that only transformants that received the IIb tail bait plasmid were strongly positive for galactosidase activity and grew on the Leucine minus the media, indicating a true positive interaction (Fig. 3). The α2 tail, IIIa tail, Lamin and null plasmid did not show galactosidase activity and failed to pass the Leucine requirement test (Fig. 3). Sequence comparison of the tail regions of the entire α integrin family showed the highest homology in 12 amino acids between IIb tail (GFFKRNRPPLEE) and αV tail (GFFKRVRPPQEE). Integrin αV tail was used as a bait to determine whether it can interact with TPI. Interestingly, we found that integrin αV displayed relatively weak binding to TPI (Fig. 4), with density scanning showing that the binding capacity is 20% of that found with the IIb tail. These data suggest that the 12 amino acids that have high homology may play an important role in the binding.

3.4. *Determination of the IIb tail peptide sequence required for TPI binding*

Site-directed mutagenesis was employed to determine which amino acid is crucial to the binding of TPI and IIb tail. The data showed that no impact to the interaction of TPI with αV tail

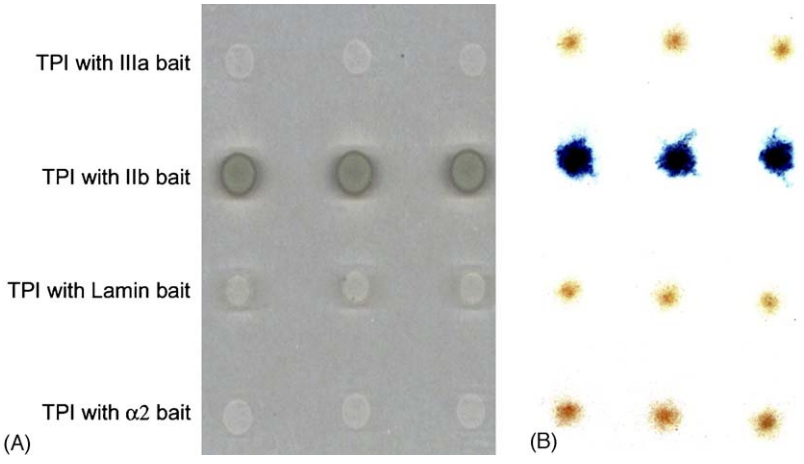

Fig. 3 – Interaction specificity of TPI with different baits. EGY48/pSH18-34 strain containing pYESTrp-TPI plasmid was transformed with different baits. Only transformants the received the IIb bait plasmid grew on the leucine minus media (LEU+) (panel A) and showed a very strong β-galactosidase activity (LacZ+) (panel B). The other baits including IIIa tail Lamin and α2 tail were negative (LEU−, LacZ−).

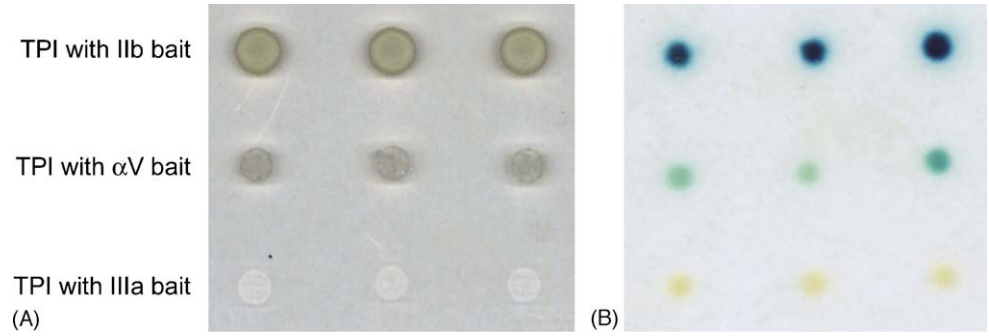

Fig. 4 – Comparison of binding capacity of IIb tail and αV tail baits with TPI in yeast. αV tail bait was transformed into EGY48/pSH18-34 strain containing pYESTrp-TPI plasmid. A relative week interaction between TPI and αV tail was detected in yeast by the grown capacity on the Leucine minus media (A) and β-galactosidase activity (B).

after mutating the following amino acids Arg (1017) and Met (1018) to Lys and Val, Val (1024) to Asn, and Gln (1028) to Leu separately (Fig. 5). However, when both Val (1024) and Gln (1028) of αV tail were mutated to Asn and Leu simultaneously, the interaction between the TPI and mutated αV tail was as strong as IIb tail (Fig. 5) suggesting the critical peptide sequence necessary for the interaction of the TPI with IIb cytoplasmic domain is GFFKRNRPPLEE.

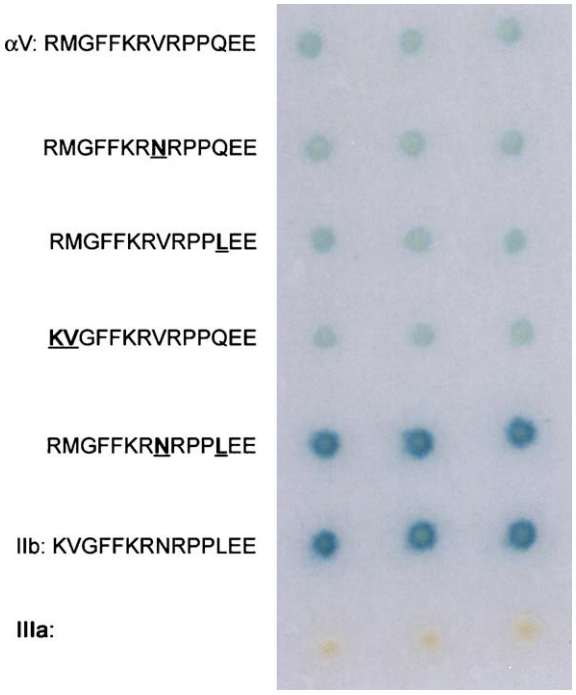

Fig. 5 – IIb tail peptide sequence required for TPI binding analyzed by mutagenesis. αV tail bait plasmid was used as target for the site-directed mutagenesis. The mutated αV tail amino acid sequences were showed in left side of the figure to their corresponding β-galactosidase activity in yeast. The interaction capacity between the TPI and αV tail was fully recovery after mutating both Val and Gln of αV tail into Asn and Leu, respectively, as showed in β-galactosidase activity (LacZ+).

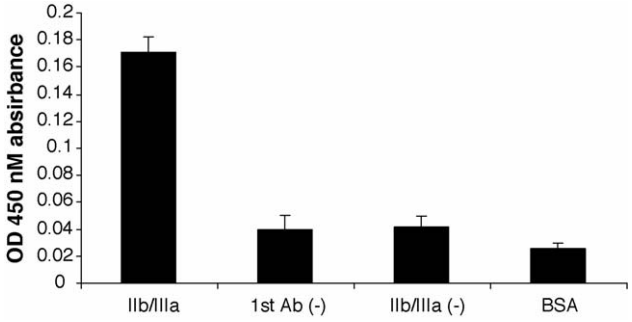

Fig. 6 – *In vitro* binding of TPI with IIb/IIIa complex. Binding of IIb/IIIa complex with immobilized TPI. BSA coated wells showed background level readings. As negative controls, TPI-coated wells incubated with IIb/IIIa complex but without anti-IIb mAb in the following steps of the assays, or incubated with anti-IIb mAb directly.

3.5. *In vitro binding of TPI to IIb/IIIa*

An ELISA assay was employed to determine whether TPI can bind to heterodimeric IIb/IIIa integrin *in vitro*. Immobilized TPI showed a significantly higher binding level to IIb/IIIa complex than did immobilized BSA (Fig. 6). In the absence of either IIb/IIIa complex or anti-IIb/IIIa antibody, the signals were similar to that obtained with BSA (Fig. 6).

3.6. *In vitro binding assays using recombinant proteins*

To corroborate the yeast two-hybrid and ELISA data, we performed an *in vitro* binding study using GST fusion protein. A single band of GST or GST-TPI fusion protein can be detected by a Western blot analysis using anti-GST monoclonal antibody (Gene Tex, Inc.) (Fig. 7A). To detect the biding of IIb with the GST-TPI fusion protein, the purified IIb/IIIa complex was allowed to interact with the immobilized GST or GST-TPI fusion proteins and was analyzed by immunoblotting with anti-IIb/IIIa antibody and anti-GST antibody simultaneously (Fig. 7B). The data clearly showed that a band of IIb was detected in the lanes of immobilized GST-TPI fusion proteins while no signal of IIb was detected in GST lane.

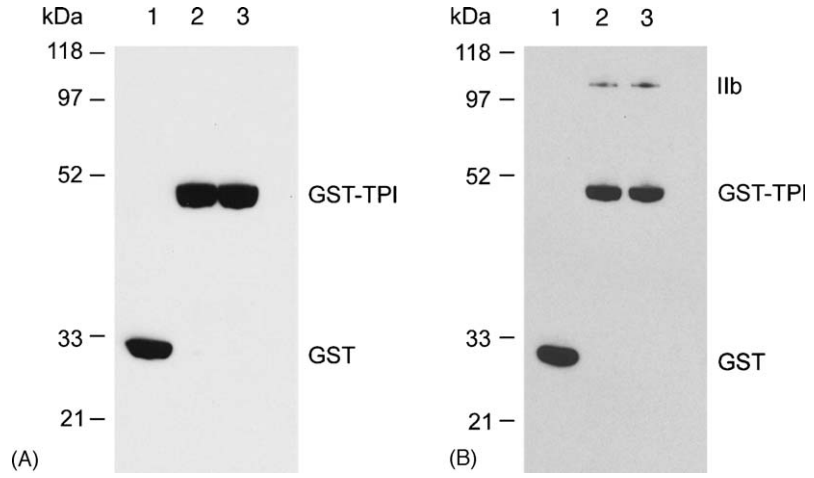

Fig. 7 – *In vitro* interaction of TPI with IIb. (A) Western blot analysis using anti-GST mAb indicates that GST and GST-TPI fusion proteins were immobilized on glutathione Sepharose beads, lanes 2 and 3 represent two different colonies expressed GST-TPI fusion proteins. (B) GST and GST-TPI fusion proteins were immobilized on glutathione Sepharose beads and purified IIb was allowed to interact with the fusion proteins. After extensive washings, bound proteins were analyzed by immunoblot with anti-GST mAb and anti-IIb mAb simultaneously.

4. Discussion

Integrins, through binding to both extracellular matrix, cytoskeletal and signaling proteins, are critical for the transmission of signals across these adhesion receptors and thus for integrin-induced spreading, migration, cell division and differentiation. The cytoplasmic domains of integrins have been implicated in the integrin affinity regulation and localization of integrins to the focal contacts. Understanding how the short cytoplasmic tails of integrins affect the functions of integrins requires identification and characterization of cellular proteins that bind, ether directly or indirectly, to integrin cytoplasmic domains. Several proteins that interact with the cytoplasmic domains of integrins have been identified [10–15]. In the present study, we have identified triosephosphate isomerase (TPI) as a novel binding protein of integrin αIIb cytoplasmic domain. This was accomplished by using αIIb cytoplasmic tail as the bait in a yeast two-hybrid system. The specificity of TPI and αIIb interaction was further confirmed by ELISA experiments using purified rabbit muscle TPI, human αIIb/β3 and an antibody raised against αIIb/β3. In addition, *in vitro* binding experiments using GST-TPI fusion protein and purified αIIb/β3 also indicated specific interaction between TPI and αIIb/β3.

The data from the present study suggest that the GFFKR motif, a common amino acid sequence found in all integrin α subunits, is necessary but not enough for the binding of IIb tail and TPI, since integrin α2 cytoplasmic tail did not show any interaction with TPI, while αV tail, which shares the highest homology with αIIb tail among the integrin α family, showed a week binding (20% compared to αIIb) to TPI in yeast two-hybrid system. Mutagenesis studies indicated that when Val (1024) and Gln (1028) of αV tail were mutated to Asn and Leu simultaneously, the interaction between αV and TPI was equivalent to that obtained with αIIb and TPI. This suggests that the TPI binding site on the αIIb cytoplasmic tail may include the 12 amino acids, which is the highest homology region between the αIIb tail and αV tail. Calreticulin, a Ca^{2+} binding protein that binds to GFFKR [15] has been shown to copurify with several integrins [18]. The results from our site-directed mutagenesis studies around this region, which includes the conserved GFFKR motif, indicated that the critical amino acids necessary for binding between TPI and IIb cytoplasmic domain are GFFKRNRPPLEE.

Triose phosphate isomerase is an enzyme involved in the energy production that is mediated exclusively by glycolysis in red cells and to a very high degree in brain cells. It catalyzes the conversion of dihydroxy acetone phosphate (DHAP) to glyceraldehydes-3-phosphate (G3P). Data published recently suggest that the glycolytic enzymes Glyceraldelhde-3-phospate dehydrogenase, triose phosphate isomerase and pyruvate kinase are components of the K^+ ATP channel macromolecular complex [20]. TPI deficiency is a rare autosomal disease and only few cases have been reported so far. The enzyme deficiency is associated with the accumulation of dihydroxy acetone phosphate (DHAP) and is characterized by hemolytic anemia, severe and progressive neuromuscular degeneration and vulnerability to infections [21]. Although it has been shown previously [22,23] that TPI can be detected in platelets, very little is known about the function of TPI in platelets. The platelets from one of the patients deficient in TPI had low levels of β-thromboglobulin and did not aggregate in response to epinephrine although the aggregation in response to ADP and collagen was normal [24,25]. In this work, we have demonstrated that the TPI was expressed in human platelets as determined by RT-PCR. Platelets have been shown to accumulate glucose from plasma and catabolize it aerobically and anaerobically. Exposure of platelets to thrombin results in the release of granules and increased activity of glycolysis and citric acid cycle. Glycolysis is activated even further when granule release is followed by aggregation. Since there is very little data on TPI and platelet function, it is difficult to compare the similarities and differences between brain TPI and platelet TPI. Based on the

data presented in this report, it is tempting to speculate that platelet TPI may play a role in the αIIB/β3 function. Nevertheless, further studies are necessary to address the physiological relevance of the interaction between TPI and αIIb tail.

Acknowledgments

Authors thank Jeremy H. Toyn for his helpful comments and kind technique support of the yeast two-hybrid system, and Yutian Zhan for assistance with the graphics.

REFERENCES

[1] Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992;69:11–25.
[2] Clark EA, Brugge JS. Integrins and signal transduction pathways: the road taken. Science 1995;268:233–9.
[3] Sastry SK, Horwits AF. Integrin cytoplasmic domains: mediators of cytoskeletal linkages and extra- and intracellular initiated transmembrane signaling. Curr Opin Cell Biol 1993;5:819–31.
[4] Schwarts MA, Schaller MD, Ginsberg MH. Integrins: emerging paradigms of signal transduction. Annu Rev Cell Dev Biol 1995;11:549–99.
[5] Horwitz A, Dugan K, Buck C, Beckerle MC, Burridge K. Interaction of plasma membrane fibronectin receptor with talin—a transmembrane linkage. Nature 1986;320:531–3.
[6] Otey CA, Pavalko FM, Burridge K. An interaction between alpha-actinin and the beta 1 integrin subunit *in vitro*. J Cell Biol 1990;111:721–9.
[7] Schaller MD, Otey CA, Hildebrand JD, Parsons JT. Focal adhesion kinase and paxillin bind to peptides mimicking beta integrin cytoplasmic domains. J Cell Biol 1995;130:1181–7.
[8] Otey C, Vasquez GG, Burridge K, Erickson B. Mapping of the alpha-actinin binding site within the beta 1 integrin cytoplasmic domain. J Biol Chem 1993;268:21193–7.
[9] Knezevic I, Leisner TM, Lam SC-T. Direct binding of the platelet integrin alphaIIbbeta3 (GPIIb-IIIa) to talin evidence that interaction is mediated through the cytoplasmic domains of both alphaIIb and beta3.. Biol Chem 1996;271:16416–21.
[10] Chang DD, Wong C, Smith H, Liu J. ICAP-1, a novel beta1 integrin cytoplasmic domain-associated protein, binds to a conserved and functionally important NPXY sequence motif of beta1 integrin. J Cell Biol 1997;138:1149–57.
[11] Hannigan GE, Leung-Hagesteijn C, Fits-Gibbon L, Coppolino MG, Radrva G, Fimus J, et al. Regulation of cell adhesion and anchorage-dependent growth by a new beta 1-integrin-linked protein kinase. Nature 1996;379:91–6.
[12] Shattil SJ, O'Toole T, Eigenthaler M, Thon V, Williams M, Babior BM, et al. Beta 3-endonexin, a novel polypeptide that interacts specifically with the cytoplasmic tail of the integrin beta 3 subunit. J Cell Biol 1995;131:807–16.
[13] Reddy KB, Gascard P, Price MG, Negrescu EV, Fox EB. Identification of an interaction between the m-band protein skelemin and beta-integrin subunits. Colocalization of a skelemin-like protein with beta1- and beta3-integrins in non-muscle cells. J Biol Chem 1998;273(52):35039–47.
[14] Kolanus W, Nagel W, Schiller B, Zeitlmann L, Godar S, Stockinger H, et al. Alpha L beta 2 integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1, a cytoplasmic regulatory molecule. Cell 1996;86:233–42.
[15] Naik UP, Patel PM, Parise LV. Identification of a novel calcium-binding protein that interacts with the integrin alphaIIb cytoplasmic domain. J Biol Chem 1997;272:4651–4.
[16] Vallar L, Melchior C, Plancon S, Drobecq H, Lippens G, Regnault V, et al. Divalent cations differentially regulate integrin alphaIIb cytoplasmic tail binding to beta3 and to calcium- and integrin-binding protein. J Biol Chem 1999;274(24):17257–66.
[17] Rojiani MV, Finlay BB, Gray V, Dedhar S. In vitro interaction of a polypeptide homologous to human Ro/SS-A antigen (calreticulin) with a highly conserved amino acid sequence in the cytoplasmic domain of integrin alpha subunits. Biochemistry 1991;30:9859–66.
[18] Leung-Hagesteijn CY, Milankov K, Michalak M, Wilkins J, Dedhar S. Cell attachment to extracellular matrix substrates is inhibited upon downregulation of expression of calreticulin, an intracellular integrin alpha-subunit-binding protein. J Cell Sci 1994;107(3):589–600.
[19] Ito H, Fukuda Y, Murata K, Kimura A. Transformation of intact yeast cells treated with alkali cations. J Bacteriol 1983;1253:163–8.
[20] Dhar-Chowdhury P, Harrell MD, Han SY, Jankowska D, Parachuru L, Morrissey A, et al. The glycolytic enzymes, glyceraldehydes-3-phpsphate dehydrogenase, triose-phosphate isomerase, and pyruvate kinase are components of the KATP channel macromolecuar complex and regulate its function. J Biol Chem 2005;280:38464–70.
[21] Ahmed N, Battah S, Karachalias N, Babaei-Jadidi R, Horanyi M, Baroti K, et al. Increased formation of methylglyoxal and prtein glycation, oxidation and nitrosation in triosephosphate isomerase deficiency. Biochim Biophys Acta 2003;1639(2):121–32.
[22] Vives-Corrons JL, Rubinson-Skala H, Mateo M, Estella J, Feliu E, Dreyfus JC. Triosephosphate isomerase deficiency with hemolytic anemia and severe neuromuscular disease: familial and biochemical studies of a case found in Spain. Hum Genet 1978;42:171–80.
[23] Skala H, Dreyfus JC, Vives-Corrons JL, Matsumoto F, Beutler E. Triose phosphate isomerase deficiency. Biochem Med 1977;18(2):226–34.
[24] Zanella A, Mariani M, Colombo MB, Borgna-Pignatti C, DeStefano P, Morgese G, et al. Triosephosphate isomerase deficiency: 2 new cases. Scand J Haemotol 1985;34:417–24.
[25] Pogliani EM, Colombi M, Zanella A. Platelet function defect in triosephosphate isomerase deficiency. Haematologica 1986;71:349–50.