Role of two conserved glycine residues in the β-propeller domain of the integrin α4 subunit in VLA-4 conformation and function

Mercedes Guerrero-Esteo, Natividad Ruiz-Velasco, Marisa Muñoz, Joaquín Teixidó*

Centro de Investigaciones Biológicas, Departamento de Inmunología, Velázquez 144, 28006 Madrid, Spain

Received 14 April 1998

Abstract The N-terminal region of the α integrin subunits is predicted to fold into a β-propeller domain. Using K562 α4 transfectants we show that mutations at α4 subunit residues Gly^{130} and Gly^{190} affect the conformation of this domain causing a reduction in the recognition of α4 by anti-α4 antibodies which map to the β-propeller. The improper α4 conformation also led to an altered association with the β1 subunit, and to a lack of α4β1 adhesion to VCAM-1 and CS-1/fibronectin, as well as an abolishment of anti-α4- and anti-β1-dependent homotypic aggregation. The total conservation of Gly^{130} and Gly^{190} among integrin α subunits suggests their importance in the correct folding of their respective β-propeller domains, and thus, in the adhesive activity of the integrins.

Key words: α4 integrin; β-propeller; Adhesion

1. Introduction

VLA-4 (α4β1) is a member of the β1 subfamily of the integrins, which mediates cell adhesion to the connecting segment-I (CS-1) region of fibronectin, and to vascular cell adhesion molecule-1 (VCAM-1) [1–5]. VLA-4, as well as other β1 integrins, can exist in different states of activation [6], and their activity can be rapidly modulated by various stimuli, including divalent cations and certain anti-integrin β1 subunit monoclonal antibodies (mAb) [6–8]. Interaction with other sequences on fibronectin [9–11], as well as with thrombospondin [12], requires VLA-4 to be activated. VLA-4 also interacts with the bacterial coat protein invasin in an activation-independent manner [13].

VLA-4 is involved in leukocyte recruitment to sites of inflammation, and it has been implicated in several inflammatory pathologies [14,15]. In addition, VLA-4 participates in the adhesion of hematopoietic progenitors to bone marrow stroma [16,17], and it is required for normal development of both T and B cells in the bone marrow [18].

A characteristic feature of integrin α subunit N-terminal domains is the presence of seven homologous repeats of approximately 60 amino acids each [19], which have been proposed to fold into a β-propeller domain, based on the presence of potential secondary structures of integrin α subunits similar to the secondary structures found in several proteins with β-propeller domains [20]. According to this model, the upper surface of the β-propeller contains the α subunit ligand-binding sites. Different epitopes involved in VLA-4-mediated cell adhesion are predicted to be present on this upper surface of the α4 β-propeller. The epitope B includes the main interaction sites with CS-1-containing fibronectin (FN) fragments and with VCAM-1, and it has been subdivided into epitopes B1 and B2, since some of the antibodies which blocked these interactions also triggered homotypic cell aggregation [21]. Using α4 murine/human chimeric constructs expressed in mammalian cells, it has been shown that epitopes B1 and B2 map to α4 residues 100–268 [22,23]. We recently reported that α4 amino acids 151–155 are important for the structure of the epitope B2 and that mutations at α4 Gln^{152} substantially impaired transfectant adhesion to a CS-1-containing fragment of fibronectin [24]. The α4 epitope A maps to the most α4 N-terminal 100 amino acids, is partially involved in cell adhesion to CS-1/FN but not to VCAM-1, and mAb against this epitope are also able to induce homotypic aggregation [21–23]. We previously showed that α4 residues Arg^{89}Asp^{90} form part of the epitope A, and are implicated in the modulation of VLA-4 adhesion to CS-1/FN [25].

The folding of the different functional epitopes in the β-propeller domain is therefore essential for the correct activity of VLA-4. The implication of small regions or individual residues in the folding of this domain remains to be solved. In the present study we have investigated the participation of two glycine residues located on the epitope B of α4 which are totally conserved among α subunits, in the folding of the α4 β-propeller domain and in the function of VLA-4.

2. Materials and methods

2.1. Cells, VLA-4 ligand proteins and antibodies

The human cell line K562 was cultured in PRMI 1640 medium supplemented with 10% fetal calf serum and antibiotics (complete medium). K562 α4 transfectants were maintained in the same medium containing 1 mg/ml of G418 (Calbiochem). The recombinant FN-H89 fragment of fibronectin which contains the CS-1 site, and recombinant soluble VCAM-1 (sVCAM-1) consisting of domains 1 to 4 were generated as previously described [24]. Monoclonal antibodies used in this study included P3X63 [26], TP1/36 (anti-CD43) [27], TS2/16 and Lia 1/2.1 (anti-VLA-β1 subunit) [28,29], SAM-1 (anti-α5) [30], and the following anti-α4: HP1/7, HP1/2, HP2/1, HP2/4 [31], and B-5G10 [32].

2.2. Site-directed mutagenesis and transfections

The α4 cDNA in Bluescript [19] was subjected to site-directed mutagenesis using the Bio-Rad Muta-Gene kit, as recommended. Oligonucleotides representing α4 bp 513–544 (for G130R) and 692–721 (for G190S) were used as primers containing selected mismatches. Transfectants expressing mutations at α4 Asn^{123}Glu^{124} have been described [24]. Base substitutions at sites of mutations raised new endonuclease restriction sites, which facilitated the identification of the mutations introduced, that were later confirmed by DNA sequencing using a Perkin-Elmer automatic DNA sequencer (Model ABI Prism). Mutated α4 inserts were excised and ligated into the pFNeo expression

*Corresponding author. Fax: +34 (1) 5627518.
E-mail: cibjt9b@fresno.csic.es

This work was supported by grant PM95-0017 from the PGC-MEC (Spain) and by a grant from SmithKline Beecham-CDTI.

PII S0014-5793(98)00576-6

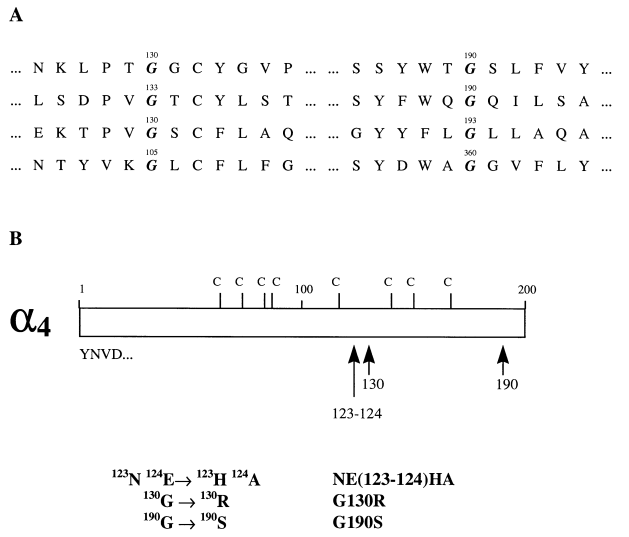

Fig. 1. Sequence alignments and mutagenesis of the integrin α4 subunit. A: Alignments of a segment at the N-terminal end of the α4 subunit which includes the residues Gly^{130} and Gly^{190}, and the corresponding on α5, αIIb and αM subunits. Glycines in bold are located at structurally equivalent loop positions in the β-propeller domain. B: Schematic diagram of the N-terminal end of α4 and site-directed mutagenesis of the α4 cDNA. Shown are the single and double mutations introduced on selected α4 residues and the designation of the α4 mutant transfectants. C denotes the cysteine residues.

vector [33,34] in *Sal*I-*Xba*I sites. For transfection, 25 μg of pFNeo vector containing mutant α4 was electroporated into 10^7 K562 cells, and after 48 h, 1 mg/ml of G418 was added to the media. G418-resistant cells were analyzed by flow cytometry using a Coulter Epics XL, and enriched for VLA-4-positive cells by immunomagnetic bead selection (Dynal Co. Norway), using the anti-α4 mAb B-5G10.

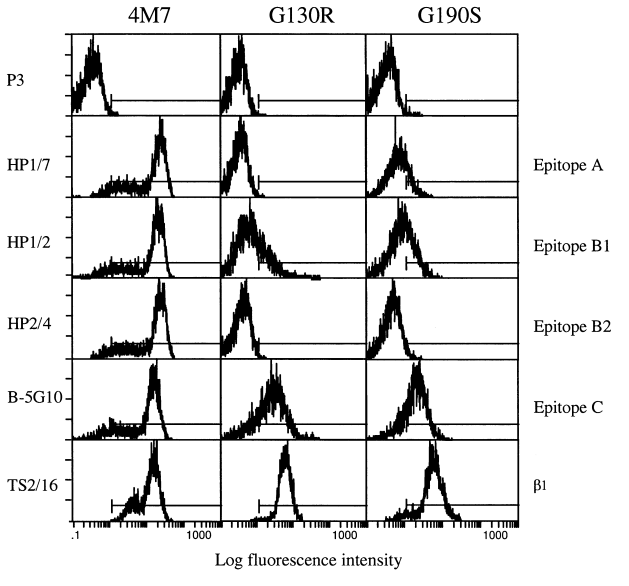

Fig. 2. Flow cytometry analysis of K562 α4 transfectants. 4M7 (wild-type α4 transfectants) and the mutant G130R and G190S were incubated at 4°C for 40 min with saturating concentrations of different anti-α4, anti-β1 or control mAb. After washing, cells were incubated at 4°C for 30 min with FITC-conjugated secondary antibody (Dako, Denmark), and finally analyzed using a Coulter Epics XL flow cytometer.

2.3. Cell labeling and immunoprecipitation

Transfectants were surface-labeled with Na[^{125}I] (Amersham Corporation, UK) using lactoperoxidase, and solubilized with 0.5% NP-40 as previously reported [34]. For immunoprecipitation, the supernatants were precleared with Protein A-Sepharose beads, followed by incubation at 4°C with antibodies. The immunocomplexes were harvested by incubation with Protein A-Sepharose beads, boiled and analyzed by SDS-PAGE using 7% polyacrylamide gels and non-reducing conditions.

2.4. Cell adhesion and aggregation assays

For cell adhesion, α4 transfectants or K562 cells were labeled in complete medium with the fluorescent dye BCECF-AM (Molecular Probes, the Netherlands), and then added in RPMI medium containing 0.4% BSA to 96-well dishes (Costar) (6×10^4 cells/well) coated with FN-H89 or sVCAM-1 (100 μl/well). After incubation for 20 min at 37°C, unbound cells were removed by three washes with RPMI medium, and adhered cells quantified using a fluorescence analyzer (CytoFluor 2300, Millipore Co.). Homotypic cell aggregation assays were performed essentially as described [35], and the degree of cell aggregation was measured at 3, 7 and 20 h in a semiquantitative manner as reported [36].

3. Results

Within the N-terminal 200 amino acids of the β-propeller α4 integrin subunit domain, two glycine residues at positions 130 and 190 are completely conserved among integrin α subunits (Fig. 1A) and are found at structurally equivalent sites in this domain, the loops connecting strands 2 and 3 of the W2 and W3 sheets, respectively [20]. According to the Peptidestructure program, the Gly^{130} is contained in a random coil sequence, while the Gly^{190} forms part of a predicted β-turn, as previously reported [37]. To investigate the importance of α4 Gly^{130} and Gly^{190} on the structure and function of VLA-4, we mutagenized both residues and the mutated full length α4 cDNAs in the expression vector pFNeo were transfected into VLA-4-negative K562 cells. The resulting geneticin-resistant transfectants were designated as indicated in Fig. 1B. We also used throughout the work the NE(123–124)HA transfectants which express α4 mutated at $Asn^{123}Glu^{124}$, that have

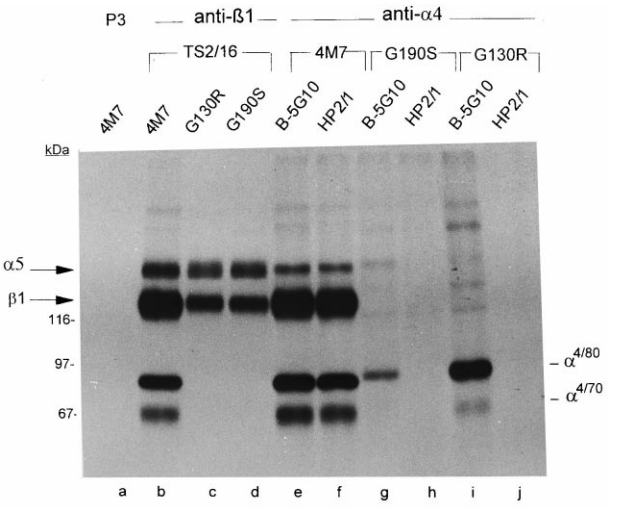

Fig. 3. Cell surface labeling of α4 transfectants. 4M7, G190S and G130R were surface iodinated, solubilized and immunoprecipitated with anti-α4 B-5G10 or HP2/1, anti-β1 TS2/16 or with control P3 mAb. Immunoprecipitates were analyzed by electrophoresis using 7% polyacrylamide gels, and bands detected by autoradiography. Indicated are the migrations of α5, $\alpha^{4/80}$, $\alpha^{4/70}$ and β1 subunits.

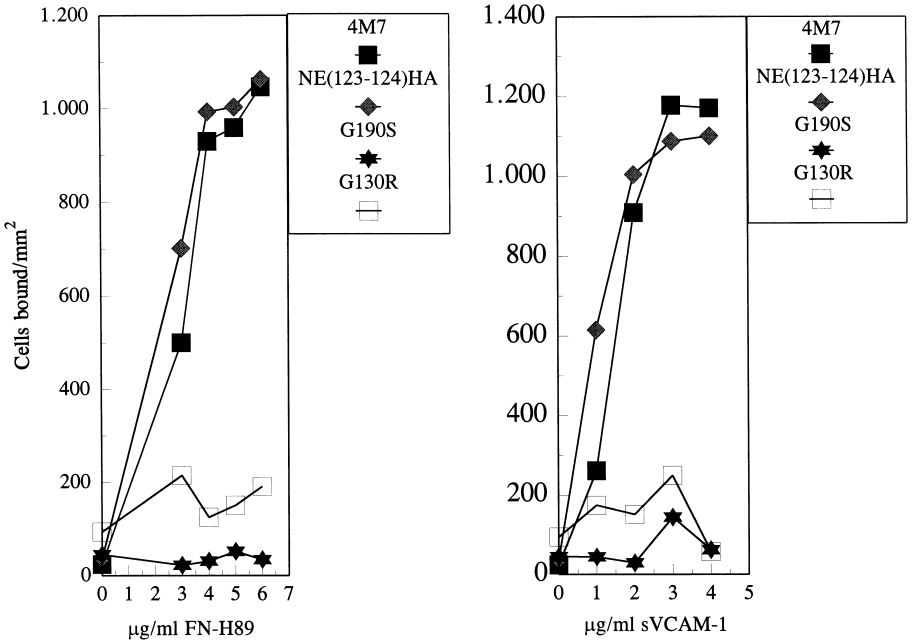

Fig. 4. Adhesion of α4 transfectants to FN-H89 and sVCAM-1. Cells were labeled with BCECF-AM and added to 96-well microtiter plates coated with increasing concentrations of FN-H89 or sVCAM-1. After 20 min incubation at 37°C, plates were washed and adhesion was quantified using a fluorescence analyzer. Each panel shows a representative result from at least four experiments and each point is the mean of triplicate determinations, with a standard deviation of $<10\%$. K562 cells showed no adhesion at any concentration of FN-H89 or sVCAM-1 tested (not shown).

previously been characterized [24]. Analysis of G130R and G190S transfectants by flow cytometry showed a very similar pattern of α4 reactivity with anti-α4 mAb which binds to different regions on the α4 molecule [21–23]. Epitopes A and B2 on α4 were clearly unreactive towards anti-α4 HP1/7 and HP2/4 mAb, respectively, while some reactivity was observed with the epitope B1 HP1/2 mAb (Fig. 2). The epitope C B-5G10 mAb reacted to a higher degree with α4 mutated at Gly^{130} than with α4 mutated at Gly^{190}. In contrast, α4 on 4M7 wild-type transfectants (Fig. 2 and [25]), as well as on NE(123–124)HA transfectant mutants (not shown, [24]), reacted comparably with all anti-α4 mAb.

The α4 subunit on the 4M7 cells showed a typical pattern of cleaved $\alpha^{4/80}$ and $\alpha^{4/70}$ proteolytic fragments expression [34], which were associated on the cell surface with the β1 subunit (Fig. 3, lanes b, e, f) as detected in cell surface iodination followed by immunoprecipitation using anti-α4 epitope C B-5G10 or epitope B1 HP2/1, or anti-β1 TS2/16 mAb. The 80 kDa and 70 kDa α4 fragments from G130R and G190S transfectants were detected in immunoprecipitates using B-5G10 but not HP2/1 mAb (Fig. 3, lanes g, h, i, j). In the same cells, the endogenous β1 subunit immunoprecipitated by TS2/16 migrated in SDS-PAGE identically to β1 from 4M7 transfectants, and the same antibody co-immunoprecipitated the α5, but not the α4 subunit in G130R and G190S cells (Fig. 3, lanes c, d). Likewise, the B-5G10 mAb failed to co-immunoprecipitate the endogenous β1 subunit in G130R and G190S cells (Fig. 3, lanes g, i), although we detected two iodinated polypeptides specifically co-immunoprecipitated by this antibody, showing an electrophoretic mobility close to endogenous β1, which were absent in the TS2/16 immunoprecipitates. Experiments of immunoprecipitation with B-5G10 of lysates from these cells, followed by Western blot using the TS2/16 anti-β1 mAb, did not reveal any associated β1 subunit (not shown), suggesting that α4β1 association is probably very weak and it might be lost during cell lysis/immunoprecipitation.

The adhesion of G130R to sVCAM-1 and to FN-H89, a

Table 1
Homotypic aggregation of wild-type and mutant α4 transfectants induced by anti-α4 mAb which recognize different epitopes on the α4 subunit

		CD43	β1	α4			
Cells	P3	TP1/36	Lia1/2	HP1/7	HP2/1	HP2/4	B-5G10
K562	0	5	0	0	0	0	0
4M7	0	5	5	4	2	4	0
NE(123–124)HA	0	5	5	4	2	4	0
G130R	0	5	1	0	0	0	0
G190S	0	5	1	0	0	0	0

K562 or transfectant K562 cells were incubated for 3 h with anti-β1 or with anti-α4 epitope A (HP1/7, B1 (HP2/1), B2 (HP2/4), or C (B-5G10). Cell aggregation was scored visually: 0, no aggregation; 1, $<10\%$ of cells aggregating; 2, 10–50% of cells aggregating; 3, 50–80% of cells aggregating; 4, $>80\%$ of cells in small aggregates; 5, $>90\%$ of cells in large and compact aggregates. No aggregation and nearly 100% aggregation were determined with the controls P3X63 and TP1/36 mAb, respectively.

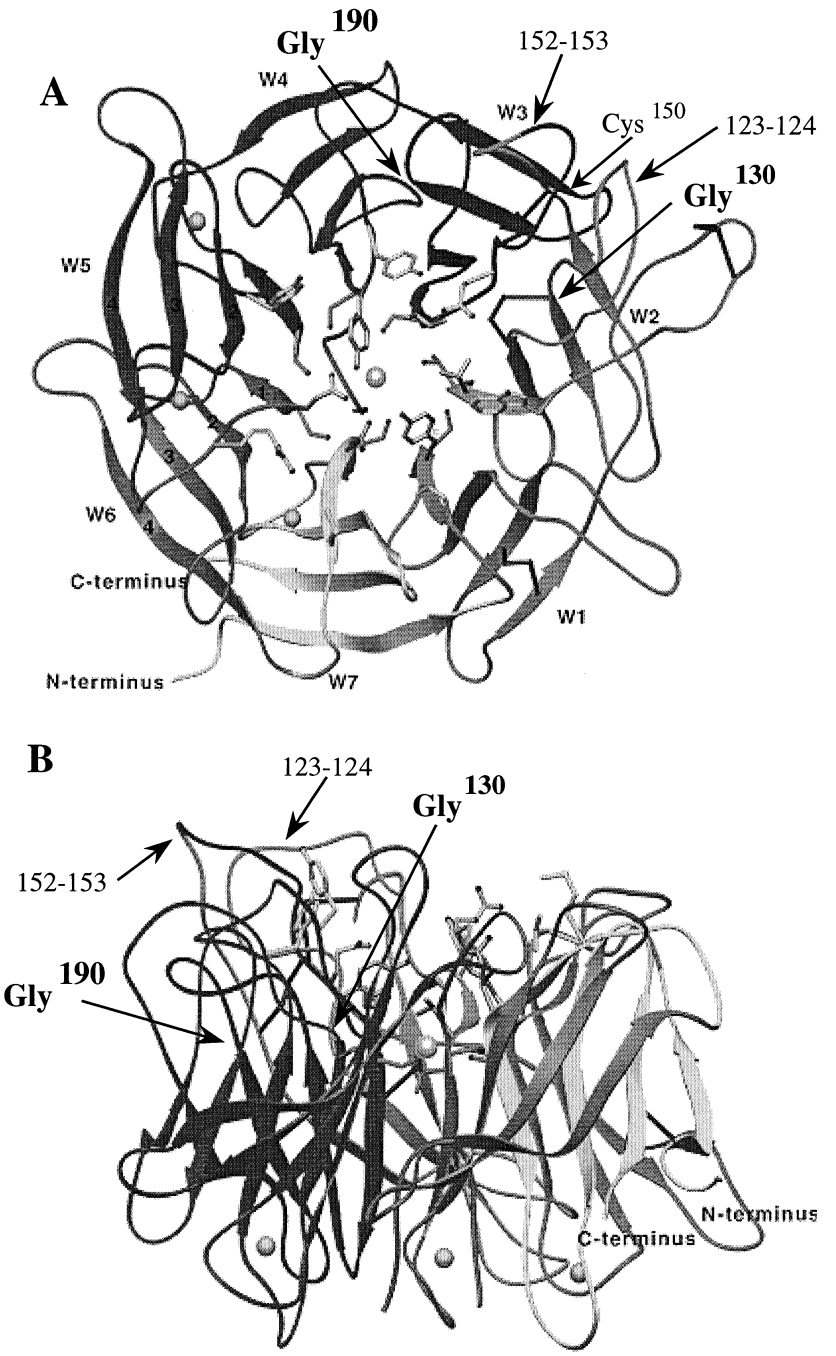

Fig. 5. Localization of Gly^{130} and Gly^{190} on the model for the integrin α4 subunit β-propeller domain (20). A: Top view. B: Side view. Also indicated are the positions for $Gln^{152}Asp^{153}$ and $Asn^{123}Glu^{124}$. The Cys^{150} is shown to help the identification of other residues.

fibronectin fragment which contains the CS-1 region, was dramatically impaired, whereas G190S cells showed a minimal adhesion to high sVCAM-1 concentrations, but not to FN-H89 (Fig. 4). In contrast, NE(123–124)HA and 4M7 transfectants adhered efficiently to increasing concentration of the ligands. The adhesion of G130R and G190S could not be restored by the stimuli TS2/16 or by Mn^{2+} ions, known to increase the affinity of VLA heterodimers for their ligands, nor by PMA, an agent that stimulates the clustering of integrins at the cell surface (not shown).

Homotypic aggregation assays showed a total absence of response to the epitope A HP1/7 and epitope B2 HP2/4 mAb by G130R and G190S cells, while high degree of aggregation was induced by these mAb in 4M7 and NE(123–124)HA transfectants (Table 1). Interestingly, G130R and G190S cells even failed to aggregate in response to the anti-β1 Lia 1/2.1 mAb. As anti-B1 mAb-mediated homotypic aggregation is dependent on the presence of cell surface VLA-4 [38], these data support the hypothesis of an altered α4β1 cell surface association. All transfectants aggregated equally well

in response to the positive control TP1/36 anti-CD43 mAb (Table 1).

4. Discussion

In this study we show that mutations at two glycines on the α4 subunit at positions 130 and 190 affect the conformation of α4, its association with the β1 subunit, and leads to a lack of VLA-4-mediated adhesive activity. Both glycines are located on structurally equivalent loops on the α4 β-propeller upper surface (Fig. 5), which has been proposed to contain key sites for interaction of VLA-4 with its ligands [20]. The total conservation of these glycines among α integrin subunits suggests that they might play an important role in the correct folding of the β-propeller domain and the whole α subunit molecules. That α4 mutated at glycines 130–190 has an incorrect conformation is supported by results showing a marked decrease in its reactivity with antibodies which recognize structurally distant regions on the β-propeller domain, such as α4 epitopes A, B1 and B2. This could be the consequence of the introduction of the substitutions at Gly^{130} and Gly^{190} which might disrupt the structure of the loops connecting strands 2 and 3 of W2 and W3 sheets, respectively. An additional mechanism contributing to the lack of recognition of the β-propeller domain by anti-α4 mAb could involve the incorrect association of α4 with the β1 subunit, similarly to what has been observed with anti-αL (CD11a) antibodies which map to the β-propeller of this α subunit [39].

Transfectant surface iodination and immunoprecipitation experiments showed that in spite of the fact that the endogenous β1 subunit is expressed on the K562 α4 transfectants in association with the α5 subunit, no endogenous β1 subunit was clearly detected in association with mutant α4. We did observe, however, two iodinated proteins on the cell surface of the mutant transfectants, specifically co-immunoprecipitated by anti-α4 mAb. Whether these two proteins correspond to anomalously processed β1 forms remains to be studied. It is well accepted that the integrin β subunits are made in excess over the α subunits, and that non-associated α or β subunits are not transported to the cell surface, but remain in cytoplasmic vesicles before they are degraded [40,41]. The present data indicate that α4 mutated at Gly^{130} and Gly^{190} is expressed on the surface of the transfectants in an altered conformation, and suggest that it might be defectively associated with unprocessed β1 forms, this association being very weak and probably lost during lysis and immunoprecipitation procedures. The addition of Mn^{2+} ions during lysis/immunoprecipitation did not influence the degree nor the quality of the recovered β1 subunits (not shown). Functional data supporting this hypothesis come from homotypic aggregation results, which show that not only aggregating anti-α4 mAb failed to induce response, but even aggregating anti-β1 mAb failed to do so, indicating that considerable conformational changes had occurred on the α4β1 heterodimer. In another previous study it was also noticed a considerable reduction in the β1 subunit co-immunoprecipitated with anti-α4 antibodies from transfectants expressing α4 mutated at Gly^{190} [37], which confirm the present results.

As expected from an altered α4β1 association and incorrect α4 conformation, transfectants expressing mutations at α4 Gly^{130} and Gly^{190} showed a dramatic impairment of VLA-4-mediated adhesion to VCAM-1 and FN-H89. The adhesion of G130R and G190S to VLA-4 ligands was not restored by stimuli which increase the affinity or the avidity of VLA heterodimers for their ligands, confirming that an important change in α4β1 conformation had occurred, and that the adhesion impairment was not due to affinity or avidity modulation. These results do not rule out that Gly^{130} and Gly^{190} could play some role in VLA-4 adhesion to its ligands. An α4 amino acid which is likely to be essential in the interaction of VLA-4 with VCAM-1 and CS-1 is Tyr^{187}, which is found on the top surface of the β-propeller [37,42]. We have also shown that α4 residues $Gln^{152}Asp^{153}$, which are located on the upper surface of the β-propeller (Fig. 5), modulate VLA-4-mediated adhesion to fibronectin [24], probably by influencing the structure of the ligand-binding site, where the Tyr^{187} is found.

The results from the present studies will contribute to a better knowledge of the relations between the structure and the function of the integrins.

Acknowledgements: We are grateful to Drs. Francisco Sánchez-Madrid and Carlos Cabañas for providing most of the antibodies used in this study, and for helpful discussions. We also thank Dr. Martin J. Humpries for the gift of fibronectin recombinants and Drs. Martin E. Hemler and Carl G. Figdor for providing different antibodies. José Luis Alonso and Reyes Tejedor are acknowledged for their help in the generation of sVCAM-1, Mario Garciá for the analyses of α4 structure, and Victoria Muñoz and Mónica Fontenla for photography.

References

[1] Wayner, E.A., Garcia-Pardo, A., Humphries, M.J., McDonald, J.A. and Carter, W.G. (1989) J. Cell Biol. 109, 1321–1330.
[2] Guan, J.L. and Hynes, R.O. (1990) Cell 60, 53–61.
[3] Mould, A.P., Wheldon, L.A., Komoriya, A., Wayner, E.A., Yamada, K.M. and Humphries, M.J. (1990) J. Biol. Chem. 265, 4020–4024.
[4] Elices, M.J., Osborn, L., Takada, Y., Crouse, C., Luhowskyj, S., Hemler, M.E. and Lobb, R.R. (1990) Cell 60, 577–584.
[5] Postigo, A., Teixidó, J. and Sánchez-Madrid, F. (1993) Res. Immunol. 144, 723–735.
[6] Masumoto, A. and Hemler, M.E. (1993) J. Biol. Chem. 268, 228–234.
[7] Arroyo, A.G., Sánchez-Mateos, P., Campanero, M.R., Martin-Padura, I., Dejana, E. and Sánchez-Madrid, F. (1992) J. Cell Biol. 177, 659–670.
[8] Kovach, N.L., Carlos, T.M., Yee, E. and Harlan, J.M. (1992) J. Cell Biol. 116, 499–509.
[9] Mould, A.P., Komoriya, A., Yamada, K.M. and Humphries, M.J. (1991) J. Biol. Chem. 266, 3579–3585.
[10] Iida, J., Skubitz, A.P.N., Furcht, L.T., Wayner, E.A. and McCarthy (1992) J. Cell Biol. 118, 431–444.
[11] Sánchez-Aparicio, P., Dominguez-Jiménez, C. and García-Pardo, A. (1994) J. Cell Biol. 126, 271–279.
[12] Yabkowitz, R., Dixit, V.M., Guo, N., Roberts, D.D. and Shimizu, Y. (1993) J. Immunol. 151, 149–158.
[13] Ennis, E., Isberg, R.R. and Shimizu, Y. (1993) J. Exp. Med. 177, 207–212.
[14] Springer, T.A. (1995) Annu. Rev. Physiol. 57, 827–872.
[15] Lobb, R.R. and Hemler, M.E. (1994) J. Clin. Invest. 94, 1722–1728.
[16] Miyake, K., Weissman, I.L., Greenberger, J.S. and Kincade, P.W. (1991) J. Exp. Med. 173, 599–607.
[17] Teixidó, J., Hemler, M.E., Greenberger, J.S. and Anklesaria, P. (1992) J. Clin. Invest. 90, 358–367.
[18] Arroyo, A.G., Yang, J.T., Rayburn, H. and Hynes, R.O. (1996) Cell 85, 997–1008.
[19] Takada, Y., Elices, M.J., Crouse, C. and Hemler, M.E. (1989) EMBO J. 8, 1361–1368.
[20] Springer, T.A. (1997) Proc. Natl. Acad. Sci. USA 94, 65–72.
[21] Pulido, R., Elices, M.J., Campanero, M.R., Osborn, L., Schiffer, S., García-Pardo, A., Lobb, R., Hemler, M.E. and Sánchez-Madrid, F. (1991) J. Biol. Chem. 266, 10241–10245.

[22] Schiffer, S.G., Hemler, M.E., Lobb, R.R., Tizard, R. and Osborn, L. (1995) J. Biol. Chem. 270, 14270–14273.
[23] Kamata, T., Puzón, W. and Takada, Y. (1995) Biochem. J. 305, 945–951.
[24] Muñoz, M., Serrador, J., Nieto, M., Luque, A., Sánchez-Madrid, F. and Teixidó, J. (1997) Biochem. J. 327, 727–733.
[25] Muñoz, M., Serrador, J., Sánchez-Madrid, F. and Teixidó, J. (1996) J. Biol. Chem. 271, 2696–2702.
[26] Lemke, H., Hammerling, G.J., Hofmann, C. and Rajewsky, K. (1978) Nature 271, 249–251.
[27] Campanero, M.R., Pulido, R., Alonso, J.L., Pivel, J.P., Pimentel-Muiños, F.X., Fresno, M. and Sánchez-Madrid, F. (1991) Eur. J. Immunol. 21, 3045–3048.
[28] Hemler, M.E., Sánchez-Madrid, F., Flotte, T.J., Krensky, A.M., Burakoff, S.J., Bhan, A.K., Springer, T.A. and Strominger, J.L. (1984) J. Immunol. 132, 3011–3018.
[29] Campanero, M.R., Arroyo, A.G., Pulido, R., Ursa, A., de Matias, M.S., Sánchez-Mateos, P., Kassner, P.D., Chan, B.M.C., Hemler, M.E., Corbi, A.L., de Landázuri, M.O. and Sánchez-Madrid, F. (1992) Eur. J. Immunol. 22, 3111–3120.
[30] te-Velde, A.A., Klomp, J.P., Yard, B.A., de-Vries, J.E. and Figdor, C.G. (1988) J. Immunol. 140, 1548–1554.
[31] Sánchez-Madrid, F., de Landázuri, M.O., Morago, G., Cebrián, M., Acevedo, A. and Bernabeu, C. (1986) Eur. J. Immunol. 16, 1342–1349.
[32] Hemler, M.E., Huang, C., Takada, Y., Schwarz, L., Strominger, J.L. and Clabby, M.L. (1987) J. Biol. Chem. 262, 3300–3309.
[33] Ohashi, P., Mak, T.W., Van den Elsen, P., Yanagi, Y., Yoshikai, Y., Calman, A.F., Terhorst, C., Stobo, J.D. and Weiss, A. (1985) Nature 316, 606–609.
[34] Teixidó, J., Parker, C.M., Kassner, P.D. and Hemler, M.E. (1992) J. Biol. Chem. 267, 1786–1791.
[35] Campanero, M.R., Pulido, R., Ursa, M.A., Rodríguez-Moya, M., De Landázuri, M.O. and Sánchez-Madrid, F. (1990) J. Cell Biol. 110, 2157–2165.
[36] Rothlein, R. and Springer, T.A. (1986) J. Exp. Med. 163, 1132–1149.
[37] Irie, A., Kamata, T., Puzon-McLaughlin, W. and Takada, Y. (1995) EMBO J. 14, 5550–5556.
[38] Sánchez-Mateos, P., Campanero, M.R., Balboa, M.A. and Sánchez-Madrid, F. (1993) J. Immunol. 151, 3817–3828.
[39] Huang, C. and Springer, T.A. (1997) Proc. Natl. Acad. Sci. USA 94, 3162–3167.
[40] Solowska, J., Edelman, J.M., Albelda, S.M. and Buck, C.A. (1991) J. Cell Biol. 114, 1079–1088.
[41] Pardi, R., Bossi, G., Inverardi, L., Rovida, E. and Bender, J.R. (1995) J. Immunol. 155, 1252–1263.
[42] Irie, A., Kamata, T. and Takada, Y. (1997) Proc. Natl. Acad. Sci. USA 94, 7198–7203.