The Collagen-binding A-domains of Integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$
Recognize the Same Specific Amino Acid Sequence, GFOGER, in Native (Triple-helical) Collagens*

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We have previously assigned an integrin $\alpha_2\beta_1$-recognition site in collagen I to the sequence, GFOGERGVEGPOPA (O = Hyp), corresponding to residues 502–516 of the $\alpha_1$(I) chain and located in the fragment $\alpha_1$(I)CB3 (Knight, C. G., Morton, L. F., Onley, D. J., Peachey, A. R., Messent, A. J., Smethurst, P. A., Tuckwell, D. S., Farndale, R. W., and Barnes, M. J. (1998) J. Biol. Chem. 273, 33287–33294). In this study, we show that recognition is entirely contained within the six-residue sequence GFOGER. This sequence, when in triple-helical conformation, readily supports $\alpha_2\beta_1$-dependent cell adhesion and exhibits divalent cation-dependent binding of isolated $\alpha_2\beta_1$ and recombinant $\alpha_2$ A-domain, being at least as active as the parental collagen. Replacement of E by D causes loss of recognition. The same sequence binds integrin $\alpha_1$ A-domain and supports integrin $\alpha_2\beta_1$-mediated cell adhesion. Triple-helical GFOGER completely inhibits $\alpha_2$ A-domain binding to collagens I and IV and $\alpha_2\beta_1$-dependent adhesion of platelets and HT 1080 cells to these collagens. It also fully inhibits $\alpha_1$ A-domain binding to collagen I and strongly inhibits $\alpha_1\beta_1$-mediated adhesion of Rugli cells to this collagen but has little effect on either $\alpha_1$ A-domain binding or adhesion of Rugli cells to collagen IV. We conclude that the sequence GFOGER represents a high-affinity binding site in collagens I and IV for $\alpha_2\beta_1$, and in collagen I for $\alpha_1\beta_1$. Other high-affinity sites in collagen IV mediate its recognition of $\alpha_1\beta_1$.

The integrins are important receptors mediating both cell-cell contact and cellular recognition of the extracellular matrix. They are heterodimers comprising an $\alpha$ and a $\beta$ chain and are classified according to the identity of the latter (1). Integrin recognition sequences have been identified in a number of matrix proteins. RGD$^X$ (where $X$ is one of several possible amino acids) occurs in a wide variety of adhesive glycoproteins and recognizes several of the integrins. In fibronectin, for example, RGD recognizes a number of integrins, including $\alpha_2\beta_1$, $\alpha_4\beta_3$, and $\alpha_5\beta_1$ (2, 3).

Integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$ are the major integrin collagen receptors (4–6). Each recognizes a variety of collagens, including collagen I, the most abundant and widely distributed of all the collagens. Recognition of collagen IV by integrin $\alpha_1\beta_1$ has been reported to involve an aspartyl residue at position 461 in the $\alpha_1$(IV) collagen chain and an arginyl residue at the same residue position in the $\alpha_2$(IV) chain (7).

Integrin $\alpha_2\beta_1$ plays an essential role in platelet adhesion to collagens in the blood vessel wall under flow conditions (8). This adhesion depends on collagen being in the triple-helical conformation (9) and is important in hemostasis, but it may also be a crucial initiator of thrombosis. Fragmentation of collagen I has indicated the presence of several $\alpha_2\beta_1$-binding sites throughout the molecule recognized by platelets (9). In particular, fragment $\alpha_1$(I)CB3 is as good as the parent collagen in supporting $\alpha_2\beta_1$-mediated platelet adhesion (9, 10). We synthesized this fragment as seven overlapping triple-helical peptides and measured their ability to mediate $\alpha_2\beta_1$-dependent cell adhesion and to bind isolated $\alpha_2\beta_1$ and the $\alpha_1$ domain derived from the $\alpha_2$ subunit, which is known to be essential for the recognition of collagen by $\alpha_2\beta_1$ (11–14). On this basis, we identified the sequence, GFOGERGVEGPOPA, corresponding to residues 502–516 of the collagen I $\alpha_1$(I) chain, as an $\alpha_2\beta_1$ binding locus in $\alpha_1$(I)CB3 (15, 16). Here we report further that $\alpha_2\beta_1$ recognition resides totally within the sequence GFOGER, that the glutamyl residue cannot be replaced by an aspartyl residue, that recognition of this sequence is entirely dependent upon the presence of a triple-helical conformation, and that the same sequence is recognized by the $\alpha_1$ A-domain of integrin $\alpha_1\beta_1$. Moreover, integrin $\alpha_2\beta_1$-mediated platelet and other cell adhesion to collagen can be completely inhibited by triple-helical GFOGER.

EXPERIMENTAL PROCEDURES

Materials—Collagen type I, for use in cell adhesion studies and solid-phase binding assays, was purified from bovine skin, following limited pepsin digestion, as described previously (9, 17). Collagen type IV from human placenta was from Sigma-Aldrich Co. Ltd., Poole, Dorset, UK.

The anti-human integrin $\alpha_1$-subunit mAb 5E8D9 was from TCS Biologicals Ltd., Botolph Claydon, Salisbury, UK; the mouse anti-human integrin $\alpha_1$ A-domain mAb 1973 (clone FB12) was from Chemicon, Harrow, UK; and the anti-(rat integrin $\alpha_1$-subunit) mAb Ha31/8 (PharMingen) was from Becton-Dickinson, San Jose, CA. Anti-(human integrin $\alpha_1$-subunit) mAb 6F1 (18) was a kind gift of Dr. B. S. Coller (Mount Sinai Hospital, New York, NY).

2 The abbreviations used are: CB, cyanogen bromide (in collagen fragment nomenclature); BSA, bovine serum albumin; GST, glutathione S-transferase; mAb, monoclonal antibody; PBS, phosphate-buffered saline; TBS, Tris-buffered saline.
Cell Culture and Adhesion—Human fibrosarcoma cells, HT 1080, were from the European Collection of Animal Cell Cultures, Porton Down, Wilts., UK. Rugli cells, derived from a rat glioma, were a kind gift from Dr. J. Gavrilovic, University of East Anglia, Norwich, UK.

Cells were maintained in Dulbecco’s Minimal Essential Medium containing 5% fetal calf serum and 100 μg/ml streptomycin, 2.5 μg/ml amphotericin, all from Sigma.

For the experiment, cells were harvested with trypsin/EDTA, fetal bovine serum was added at 1:1 v/v, and cells were recovered by centrifugation. After four washes with Dulbecco’s phosphate-buffered saline (Ca2+/Mg2+-free PBS), the pellet was resuspended at a concentration of 0.3 x 106 cells/ml in adhesion buffer (TBS plus glucose, 0.9 g/l), containing 1 mM MgCl2 or 2 mM EDTA, as necessary. Immunol-2 multi-well plates were coated with collagen or peptide, normally with 100 μl of a 10 μg/ml solution in 0.01 M acetic acid for 1 h at 20 °C, blocked with BSA (100 μl of a 1 mg/ml solution in PBS), and then washed four times with PBS. 100 μl of cell suspension were then added and adhesion measured at 20 °C (to maintain the peptides in a triple-helical configuration) at times specified later (see “Results”). Unattached cells were counted using a Coulter Counter (model ZF) and adhesion was calculated as the number of adherent cells expressed as a percentage of the total cell count. Assays were undertaken in triplicate, and the data were expressed as the mean ± S.D. BSA-coated wells were used to determine non-specific attachment. Adhesion was considered to be that resulting from the interaction of the cell with collagen, reaching a maximum value of about 80% within 30 min. Rugli cells initially attached rapidly but only reached a maximum adhesion, typically about 65%, after 90 min. Results of experiments are expressed on the basis of a value of 100% for collagen. Data presented are representative of three similar experiments. Cells were preincubated with antibody or peptide, when testing for inhibition, for 15 min.

Platelet Adhesion—Adhesion was determined colorimetrically (19). Washed platelets, from platelet-rich plasma as described previously (20), were suspended routinely at 1.0–1.5 x 106/ml in adhesion buffer, TBS containing 0.1% BSA (Sigma A7638), and the suspension rested for 30 min prior to use. MgCl2 or EDTA were added to 2 mM as required. When testing mAbs or peptides for inhibitory activity, the platelet suspension was preincubated with antibody for 15 min. Immunol-2 96-well plates were coated with collagen or peptide, routinely at 10 μg/ml, and then blocked with BSA (Sigma A7638). 50 μl of platelet suspension were added to each well, and plates were incubated for 60 min at 20 °C (to ensure retention of triple-helical conformation). Unbound platelets were then discarded, and the wells were washed three times with 200 μl of adhesion buffer. 150 μl of lysis buffer (0.1 mM citrate, pH 5.4, containing 6.1% Triton X-100 and 5 mM p-nitrophenol phosphate) was added to each well. Reaction was terminated after 60 min by addition of 100 μl of 2 mM NaOH, and plates were read at 405 nm using an automated plate reader (Emax; Molecular Devices). Assays were made in triplicate and the results expressed as the mean ± S.D., relative to a value of 1.0 for collagen. Data are representative of three repeat experiments. In a typical experiment, a platelet concentration of 2.5 x 107/ml gave an Amax for collagen 1 as substrate. The results corresponded to adhesion of about 15% when expressed as a fraction of the number of cells applied.

Integrin α1β2—The integrin was extracted from human platelet membranes and purified by affinity chromatography on collagen-Sepharose as described previously (15). Homogeneity was established by polyacrylamide gel electrophoresis, and identification as α1β2 was by immunoprecipitation and Western blotting (15). Protein concentration was determined with a Micro BCA Protein Assay reagent (Pierce and Warriner (UK) Ltd., Chester, UK). The protein was biotinylated using an Amersham Pharmacia Biotech ECL biotinylation module, according to the manufacturer’s instructions. The suitability of the biotinylated product for use in solid-phase assays was demonstrated in our earlier work (15, 16).

Recombinant Integrin α1 and α2 A-Domains—The production of recombinant human α1 and α2 A-domains and their isolation as A-domain glutathione S-transferase (GST) fusion proteins has been described previously (13, 21). The suitability of these materials for use in solid-phase binding assays has been established in our earlier work (13, 15, 16, 21).

Solid-Phase Binding Assays—Assays were performed as described previously (13, 15, 16, 21). Briefly, 96-well MaxiSorp plates (Nunc) were coated with collagen or peptide at 10 μg/ml for 1 h, blocked for 30 min with 200 μl of 50 mg/ml BSA (Sigma A4503) in TBS, and then washed three times with TBS containing 1 mM MgCl2 and 1 mg/ml BSA (Sigma A7638). Ligand dissolved in 100 μl of TBS containing 1 mg/ml BSA (A7638) and 2 mM MgCl2 or 5 mM EDTA as required, either biotinylated α1β2 (1 μg/ml) or A-domain fusion protein (5 μg/ml) was applied to wells. Where required, antibody or peptide, when testing for inhibition, was added to ligand solutions 15 min prior to their application to wells. Plates were incubated for 90 min at 20 °C, then washed three times as above. Bound biotinylated α1β2 was detected with streptavidin-horseradish peroxidase (1:1500 in TBS), bound A-domain fusion protein with horseradish peroxidase-linked anti-GST antibody (Sigma A1500) in 1/500 in TBS. Color was subsequently developed using a TMB substrate kit (Pierce) according to the instructions of the manufacturer and plates read at 450 nm with the E-max plate reader. Assays were performed in triplicate and results expressed as the mean ± S.D., relative to a value of 1.0 for collagen. Data are shown as representative of at least three repeat experiments.

Peptides—Synthesis of the seven overlapping peptides CB3(I)-1 to 7, based on the sequence of the collagen type I fragment α1(1)CB3, together with peptide CB3(I)-5/6-GAR containing the overlap sequence (FGOFGERGEGPOGA) between CB3(I)-5 and -6, except that the glutamyl residue in the triplet GER has been replaced by an alanyl residue, has been described by us earlier (15). The peptide CB3(I)-5/6-GPP (formerly designated 5/6-HYP2) containing the overlap sequence within repeat GPPs, rather than repeat GPOs as in the above peptides, has also been described in our earlier work (16). Peptides made in the current study are listed in Table I. Sequences of interest were incorporated within repeat GPP triplets (to ensure triple-helicity) rather than repeat GPO triplets because the platelet collagen receptor glycoprotein VI does not recognize the GPP sequence (16), and therefore platelet adhesion would not be complicated by the occurrence of glycoprotein VI-mediated adhesion. Peptides were synthesized as C-terminal amides on TentaGel R RAM resin in a PerSeptive Biosystems 9050 Plus Peptide Synthesizer exactly as described in our earlier studies (15, 16). Peptides were purified by reverse-phase high performance liquid chromatography on a column of Vydatec 219TP101522 using a linear gradient of 5–45% acetonitrile in water containing 0.1% trifluoroacetic acid. Fractions containing homogeneous product were identified by analytical high performance liquid chromatography on a column of Vydatec 219TP754, pooled, and freeze-dried. All peptides were found to be of the correct theoretical mass by mass spectrometry. The triple-helical stability of each peptide was assessed by polarimetry as described previously. The melting temperature (Tm) was calculated by fitting a theoretical melting equation to the melting curve by nonlinear regression.3 Values are given in Table I.

RESULTS
Identification of FGOFGER as an α1β2-recognition Sequence—In our previous study, we showed that platelet and HT 1080 cell adhesion to the collagen I sequence FGOFGERGEGPOGA, incorporated within repeat GPP triplets to ensure a triple-helical conformation (peptide CB3(I)-5/6-GPP, Table I), was divalent cation-dependent and totally mediated by α1β2. The sequence bound both isolated α1β2 and recombinant α2 A-domain, and binding was inhibited by both EDTA and anti-α1 mAbs. The GER triplet appeared essential for activity because replacement of the glutamyl or arginyl residue by alanine eliminated recognition of α1β2 (15, 16). In the present study, we have examined the effect of removal from the C terminus of CB3(I)-5/6-GPP of G (peptide desGPP-GPP in Table I), GPPGGA (desGPPGGA, Table I), and GVEGPOGPA (peptide GFOGER-GPP, Table I) and of G from the N terminus (peptide desFGO-GPP, Table I). Removal of the C-terminal sequences had no significant effect on the level of adhesion of platelets or HT1080 cells nor on the extent of C-terminal sequences had no significant effect on the level of adhesion of platelets or HT1080 cells nor on the extent of triple-helical conformation (peptide CB3(I)-5/6-GPP, Table I), GPPGGA (desGPPGGA, Table I), and GVEGPOGPA (peptide GFOGER-GPP, Table I) and of G from the N terminus (peptide desFGO-GPP, Table I). Removal of the C-terminal sequences had no significant effect on the level of adhesion of platelets or HT1080 cells nor on the extent of triple-helical conformation (peptide CB3(I)-5/6-GPP, Table I), GPPGGA (desGPPGGA, Table I), and GVEGPOGPA (peptide GFOGER-GPP, Table I) and of G from the N terminus (peptide desFGO-GPP, Table I). Removal of the C-terminal sequences had no significant effect on the level of adhesion of platelets or HT1080 cells nor on the extent of binding of the isolated intact α1β2 integrin or the recombinant α2 A-domain. However, removal of GFO caused a marked loss of activity in every case (Fig. 1). This indicates that α2β1 recognition resides entirely within the sequence FGOFGER. Like the parent peptide CB3(I)-5/6-GPP, peptide GFOGER-GPP supported platelet adhesion and exhibited α2β1 binding as good as that to collagen I, whereas binding of the α2 A-domain was consistently higher than that to the collagen (Figs. 1 and 2). Platelet adhesion and the binding of α2β1 and α2 A-domain, as for collagen, was divalent cation-dependent and strongly inhibited.3 C. G. Knight, D. J. Onley, and K. Smith, unpublished data.
A Collagen I Sequence Recognized by Integrins α₁β₁ and α₂β₁

Peptide CB3(I)-5/6-GPP containing the 15-mer α₂β₁-recognition site, shown in bold, corresponding to residues 502–516 of the α1(I) chain of type I collagen has been described by us previously (16). Other peptides listed were synthesized in the present study. The sequence of interest (shown in bold) was incorporated within repeat GPP triplets, as shown, to ensure triple helicity. A cysteine was incorporated at either end to allow cross-linking as desired.

### Table I

**Peptide sequences**

Peptide CB3(I)-5/6-GPP, desGPA-GPP, desGPOGPA-GPP, desGF-O-GPP and GFOGER-GPP. Platelet adhesion was measured as absorbance at 405 nm, α₂β₁-binding and α₂ A-domain binding at an absorbance of 450 nm. HT 1080 cell adhesion (at 60 min) was recorded as percent adhesion (vertical axis). All values relate to a value for collagen of 1.0 (absorbance) or 100% (HT 1080 adhesion). Results are the mean of triplicate determinations ± S.D. Where error bars are absent, they were too close to show.

| Peptide            | Sequence                                                                 | Tₐm   |
|--------------------|--------------------------------------------------------------------------|-------|
| CB3(I)-5/6-GPP     | GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC                                    | 42.7 ± 1.1 |
| desGPA-GPP         | GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC                                    | 42.5 ± 0.6 |
| desGPOGPA-GPP      | GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC                                    | 38.6 ± 0.6 |
| desGF-O-GPP        | GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC                                    | 46.7 ± 0.6 |
| GFOGER-GPP         | GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC                                    | 43.9 ± 0.7 |
| GFOGDR-GPP         | GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC                                    | 40.1 ± 0.4 |
| GFOGEK-GPP         | GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC(GPP)₅GPC                                    | 37.9 ± 0.3 |
| GFOGER-GAP         | GAC(GAP)₅GAC                                                              |       |

**Fig. 1. Reactivity of peptides CB3(I)-5/6-GPP, desGPA-GPP, desGPOGPA-GPP, desGF-O-GPP and GFOGER-GPP.** Platelet adhesion was measured as absorbance at 405 nm, α₂β₁-binding and α₂ A-domain binding at an absorbance of 450 nm. HT 1080 cell adhesion (at 60 min) was recorded as percent adhesion (right vertical axis). All values relate to a value for collagen of 1.0 (absorbance) or 100% (HT 1080 adhesion). Results are the mean of triplicate determinations ± S.D. Where error bars are absent, they were too close to show.

**Fig. 2. Integrin recognition by peptide GFOGER-GPP and related peptides.** A, integrin α₂β₁-dependent platelet adhesion; B, binding of α₂ A-domain. Assays were in the presence of Mg²⁺ unless EDTA is indicated. mAb 6F1 was employed at 2 μg/ml. Results are expressed as a mean of three determinations ± S.D. Missing values were too low to reproduce; missing error bars were too close to show.

### Recognition of α₁β₁—The A-domain of the α₁ subunit of integrin α₁β₁ is known to be essential for α₁β₁-binding to collagen, including collagen I (21, 22), just as the α₂ A-domain, binding of α₂β₁-collagen binding (at 60 min) was recorded as percent adhesion (right vertical axis). All values relate to a value for collagen of 1.0 (absorbance) or 100% (HT 1080 adhesion). Results are the mean of triplicate determinations ± S.D. Where error bars are absent, they were too close to show.

As previously shown for the α₂ A-domain, binding of α₂ A-domain to peptide CB3(I)-5/6-GPP or peptide GFOGER-GPP, as to collagen (21, 23), was divalent cation-dependent and was inhibited by antibody directed to the A-domain (Figs. 4, 5, and 6). Binding of the α₁ A-domain to either peptide, while blocked by anti-α₁, A-domain, was unaffected by anti-α₂ A-domain mAb. Conversely, binding of α₂ A-domain was inhibited by anti-α₂ A-domain mAb, but not by the anti-α₁, A-domain antibody. Results for CB3(I)-5/6-GPP are shown in Fig. 5.

Confirmation of the recognition of GFOGER by α₁ A-domain was obtained with Rugli cells which express the integrin α₁β₁ that mediates their adhesion to collagen (24). Testing the α1(I)CB3-based peptides, CB3(I)-1 to -7, we found, as for α₂β₁-mediated cell adhesion (15, 16), preferential adhesion to CB3(I)-5 and -6 that was divalent cation-dependent and oc-
Good adhesion, as good as to collagen, occurred to CB3(I)-5/6-GPP, confirming the presence of an $\alpha_1\beta_1$-recognition site in the overlap sequence, GFOGERGVEGPOGPA (Fig. 7). Adhesion to collagen was completely blocked by the anti-$\alpha_1$ subunit mAb Ha31/8 (tested at 15 $\mu$g/ml), and that to CB3(I)-5/6-GPP was inhibited by 80% (data not shown). The location of the $\alpha_1\beta_1$ recognition site to GFOGER was substantiated on the basis that adhesion to GFOGER-GPP was as good as that to collagen or CB3(I)-5/6-GPP (Fig. 6), and the relatively poor adhesion to desGFO-GPP, and to CB3(I)-5/6-GAR in which the glutamyl residue in the GER triplet has been replaced by an alanyl residue (Fig. 7). Adhesion of Rugli cells to GFOGER-GPP, as to collagen, was divalent cation-dependent and inhibited by the anti-$\alpha_1$ mAb Ha31/8 (data not shown).

### Structural Specificity of GFOGER for $\alpha_1\beta_1$ and $\alpha_2\beta_1$ Recognition

The conservative replacement of the glutamyl residue in GFOGER-GPP by an aspartyl residue caused a marked loss of integrin recognition. GFOGDR-GPP (see Table I) failed to support platelet adhesion (Fig. 2A), or adhesion of HT 1080 cells to collagen and peptide CB3(I)-5/6-GPP. Assays were in the presence of Mg$^{2+}$, antibody was either absent (–AB) or included as anti-$\alpha_1$ mAb 5E8D9 (15 $\mu$g/ml) or anti-$\alpha_2$ mAb 6F1 (2 $\mu$g/ml), as indicated. Results are shown as a mean of three determinations ± S.D. Missing values were too low to reproduce. Missing error bars were too close to show.
(data not shown), nor was the peptide able to bind \( \alpha_2 \beta_1 \) (data not shown) or \( \alpha_2 \) or \( \alpha_1 \) A-domain (Figs. 2B and 6). Rugli cell adhesion to the peptide was only around one-quarter of that to collagen I (data not shown). On the other hand, replacement of arginine by lysine (peptide GFOGEK-GPP; Table I) caused only a partial loss (about 50\%) of cell adhesion (Fig. 2A) and integrin binding (data not shown) although A-domain binding was largely eliminated (see Figs. 2B and 6).

**Requirement for the Triple-helical Conformation—**Peptide GFOGER-GAP (Table I) contains the integrin A-domain recognition sequence GFOGER in repeat GAP rather than GPP triplets. As anticipated, polarimetry indicated the absence of any triple-helical structure. In marked contrast to GFOGER-GPP, the peptide failed to support adhesion of platelets (Fig. 2A) or HT 1080 cells (data not shown) and exhibited complete absence of binding of isolated \( \alpha_2 \beta_1 \) (data not shown) or \( \alpha_1 \) (Fig. 6) and \( \alpha_2 \) A-domains (Fig. 2B), emphasizing the crucial role of the triple-helical conformation for recognition of \( \alpha_1 \beta_1 \) and \( \alpha_2 \beta_1 \) A-domains by collagen.

**Inhibition of Cell Adhesion by Peptide GFOGER-GPP—**GFOGER-GPP was a potent inhibitor of platelet adhesion to collagens I and IV with an IC\(_{50}\) for collagen I of approx. 75 \( \mu \)g/ml or 7 \( \mu \)M; \( M_r \) 11,112 (Fig. 8A), and of \( \alpha_2 \beta_1 \) and \( \alpha_2 \) A-domain binding to these collagens (IC\(_{50}\) ~30 \( \mu \)g/ml or 3 \( \mu \)M; Fig. 8, B–D), confirming the identity of GFOGER as an \( \alpha_2 \beta_1 \) integrin recognition site and establishing the crucial importance of this sequence as a platelet \( \alpha_2 \beta_1 \)-binding locus in collagens I and IV. The peptide also fully inhibited \( \alpha_2 \beta_1 \)-mediated adhesion at 30 min of HT 1080 cells to collagens I and IV with an IC\(_{50}\) of ~450 \( \mu \)g/ml or 40 \( \mu \)M (data not shown). GFOGER-GAP, tested up to 3 mg/ml (300 \( \mu \)M; \( M_r \) 10,176) exhibited no inhibitory activity, confirming the essential requirement of the triple-helical conformation for recognition of GFOGER (Fig. 8, A and B).

GFOGER-GPP also totally inhibited \( \alpha_1 \) A-domain binding to collagen I (Fig. 8C), and in accord with this, \( \alpha_2 \beta_1 \)-dependent adhesion (at 30 min) of Rugli cells to this collagen was strongly inhibited (80\%) by the peptide (IC\(_{50}\) around 40 \( \mu \)M; data not shown). By contrast, GFOGER-GPP had little effect on \( \alpha_1 \) A-domain binding to collagen IV (Fig. 8D) or the attachment of Rugli cells, even when tested up to 3 mg/ml (275 \( \mu \)M).

**DISCUSSION**

We previously identified the sequence GFOGERGVEGPOGPA, residues 502–516 of the \( \alpha_1 \)I chain of collagen I, as an integrin \( \alpha_2 \beta_1 \) recognition sequence (15, 16). Here we show that recognition resides within the six-residue sequence GFOGER. There appears to be an absolute requirement for the glutamyl residue because even the conservative replacement with an aspartyl causes a complete loss of recognition. The requirement for a glutamyl residue is of particular interest in view of crys-
tallographic evidence that predicts recognition by the α2β1 A-domain of a glutamate sidechain in collagen (25). In the peptide desGFO-GPP, GFO is in effect replaced by GPP (see Table I), and this is shown here to be inactive. We have also found that a peptide akin to desGFO-GPP, but in which the repeat GPPs are replaced by repeat GPOs, so that in effect GFO becomes GPO, is also inactive. This indicates that it is the identity of the residue in the X position in the GXY triplet, namely the phenylalanyl residue in the GFO triplet, that is crucial for recognition.

Our studies here have shown that the sequence GFOGER is recognized equally well by both α1 and α2 A-domains and that the sequence can support both α1β1- and α2β1-dependent cell adhesion. In each case, recognition requires the same structural features. In particular, the glutamyl residue is essential for recognition of both A-domains. It is of interest that the α1 A-domain has a crystal structure very similar to that of the α2 A-domain (26). Recognition of GFOGER by two different integrins, α1β1 and α2β1, is perhaps akin to the recognition of the same RGD sequence in fibronectin by at least eight different integrins, including α2β1, α1β1, αβ2, and αβ2β1 (2, 3).

Interestingly, substitution of R by K in GFOGER led to a loss of recognition of the α1 A-domain, but α2β1-mediated cell adhesion was only reduced by around one-half. The reason for this is unclear but may suggest that structural requirements for recognition of the isolated A-domain are more stringent than those required for recognition of the domain within the intact integrin located in the cell membrane.

Triple-helical GFOGER is a potent inhibitor of α2β1-mediated adhesion of platelets and HT1080 cells to collagen I and fully inhibits binding of the isolated α2β1 integrin and the recombinant α2 A-domain to this collagen. The same holds true for collagen IV, and it is of interest that the major cell-binding domain of collagen IV possessing both α1β1- and α2β1-binding sites (7) contains a GFOGER sequence in the α1(IV) chain (see residues 405–410 in Ref. 7; Ref. 27). The collagen I fragment α1(1)CB3 containing the GFOGER sequence under consideration here supports α2β1-mediated platelet adhesion as well as the parent collagen and better than other collagen I-derived fragments (9, 10). Our results indicate that GFOGER is a major α2β1 recognition site in collagens and is responsible for their interaction with cells via the integrin α2β1. However, some collagen I fragments, for example, bovine α1(1)CB7 and α1(1)CB8, support some α2β1-mediated platelet adhesion (9) despite the absence of the sequence GFOGER (28). Furthermore, α2β1-dependent cell adhesion to bovine collagen III is totally inhibited by the peptide GFOGER-GPP although the GFOGER sequence is not present in collagen III (28). Presumably, other sequences are able in some measure to support α2β1-mediated cell adhesion.

Binding of α1 A-domain to collagen I, like that of the α2 A-domain, is fully inhibited by triple-helical GFOGER-GPP, and α1β1-dependent adhesion of Ruggi cells to this collagen is mostly prevented, suggesting that GFOGER (or sequences of similar affinity) play a major role in mediating α1β1-dependent cell adhesion to collagen I. However, α1 A-domain binding to collagen IV is only relatively poorly inhibited by GFOGER-GPP, which is in accord with our finding that Ruggi cell adhesion to collagen IV is not inhibited by GFOGER-GPP. This indicates that a sequence of higher affinity than GFOGER must mediate α1β1-dependent cell adhesion to collagen IV, despite the presence of GFOGER, and this accords with the data of others (7) that α2β1-binding to collagen IV involves the residues aspartyl 461 in the α1(IV) chain and arginyl 461 in the α2(IV) chain.

In summary, we find that α1 and α2 A-domains each recognize the sequence GFOGER, and our data are consistent with the proposal that GFOGER is responsible in large part for α2β1-dependent cell recognition by collagens I and IV and, conceivably, other collagens. We find too that the sequence may mediate α1β1-dependent cell adhesion to collagen I but plays no significant role in such adhesion to collagen IV.

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