A Gly → Ser Change Causes Defective Folding in Vitro of Calcium-binding Epidermal Growth Factor-like Domains from Factor IX and Fibrillin-1*

(Received for publication, September 4, 1997, and in revised form, December 16, 1997)

Pat Whiteman‡, A. Kristina Downing§†, Rachel Smallridge§, Peter R. Winship**, and Penny A. Handford¶‡‡

From the ‡Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, the §Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, and the ¶‡‡Division of Molecular and Genetic Medicine, University of Sheffield, Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF, United Kingdom

The calcium-binding epidermal growth factor-like (cbEGF) domain is a common motif found in extracellular proteins. A mutation that changes a highly conserved Gly residue to Ser in this domain has been identified both in the factor IX (FIX) and fibrillin-1 genes, where it is associated with relatively mild variants of hemophilia B and Marfan syndrome, respectively. We have investigated the structural consequences in vitro of this amino acid change when introduced into single cbEGF domains from human FIX (G60S) and human fibrillin-1 (G1127S), and a covalently linked pair of cbEGF domains from fibrillin-1. High pressure liquid chromatography analysis, mass spectrometry, and 1H NMR analysis demonstrate that wild-type cbEGF domains purified in the reduced form and refolded in vitro adopt the native fold. In contrast, the Gly → Ser change causes defective folding of FIX and fibrillin-1 cbEGF domains. However, in the case of the factor IX mutant domain, a Ca2+-dependent change in conformation, identified by NMR in a proportion of the refolded material, suggests that some material refolds to a native-like structure. This is consistent with enzyme-linked immunosorbent assay analysis of FIX G60S from a hemophilia B patient Oxford d2, which demonstrates that the mutant protein is partially recognized by a monoclonal antibody specific for this region of FIX. NMR analysis of a covalently linked pair of fibrillin cbEGF domains demonstrates that the C-terminal domain adopts the native epidermal growth factor fold, despite the fact that the adjacent mutant domain is misfolded. The implications of these results for disease pathogenesis are discussed.

The calcium-binding epidermal growth factor-like (cbEGF)$^1$ domain is a module found in extracellular proteins with widely different functions such as extracellular matrix architecture, control of blood coagulation, cholesterol uptake and cell fate determination (1). It is defined by a consensus sequence (D/N-X-(D/N)-(E/Q)-Nm-(D/N*)-Nm-(Y/F) (where m and n are variable, and * indicates a potential β-hydroxylation site) (2–4), and 6 conserved cysteine residues associated with all epidermal growth factor domains which disulfide bond in a 1–3, 2–4, 5–6 arrangement. The geometry of calcium binding is pentagonal bipyramidal (5), and in human fibrillin-1 calcium is thought to perform a key structural role in stabilizing the linkage between contiguous cbEGF domains (6). The importance of the cbEGF domain is highlighted by the identification of mutations that cause amino acid changes in this domain in patients with hemophilia B (7), Marfan syndrome (8), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (9), familial hypercholesterolaemia (10) (defects in FIX, fibrillin-1, notch 3, and the low density lipoprotein receptor, respectively), and protein S deficiency (11).

Of all the missense mutations identified so far in cbEGF domains, one of the most interesting changes a highly conserved Gly residue to a Ser. This has been identified in the human FIX gene (G60S, over 50 reported cases) and is associated with mild hemophilia B (12), and also in the human fibrillin-1 gene (G1127S), where it predisposes to ascending aortic aneurysm and dissection in later life (13). From our structural knowledge of cbEGF domains, one would predict that this mutation might cause defective folding, since the conserved Gly is located in a turn at the end of a two stranded β-sheet (see Fig. 1), the main secondary structure feature of this type of domain (5, 6). This study investigates the structural consequences of these Gly → Ser amino acid changes introduced into single cbEGF domains from factor IX and fibrillin-1, and into a covalently linked pair of cbEGF domains (cbEGF domains 13 and 14) from fibrillin-1. We demonstrate in vitro that a Gly → Ser change does result in defective folding when introduced into single cbEGF domains from FIX and fibrillin-1, but in the case of FIX mutant cbEGF domain, a proportion of this material has a calcium-dependent conformation, similar to the wild-type domain. We show that this is consistent with the in vivo situation since plasma FIX from the hemophilia B patient Oxford d2 (14), containing the G60S change, shows significant reactivity toward a FIX monoclonal antibody specific for this region of FIX (12). When the Gly → Ser mutation is introduced into a covalently linked pair of fibrillin-1 cbEGF domains, the C-terminal domain adopts the native fold, despite the fact that the adjacent mutant domain is misfolded. The possible consequences of these results for the...
expression of mutant proteins with Gly → Ser amino acid changes in cbEGF domains are discussed.

**EXPERIMENTAL PROCEDURES**

**Chemical Synthesis of Single cbEGF Domains from Fibrillin-1—**Two peptides (residues 1112–1154 of human fibrillin-1) were synthesized by use of conventional Fmoc (N-9-fluorenylmethoxycarbonyl) chemistry on an Applied Biosystems 430A synthesizer. One peptide corresponded to the 13th cbEGF domain, and the second contained a G1127S amino acid change. The peptides were deprotected and cleaved from the resin by treatment with a mixture of 87.5% trifluoroacetic acid, 5% water, 2.5% 1,2-ethanedithiol, 2.5% thioanisole, and 2.5% ethylmethylsulfide (4 ml/100 mg of resin) for 3 h in a sonicating water bath. Resin was removed by filtration, and peptides precipitated with tertiary butyl methyl ether. The precipitate was washed with diethyl ether, dissolved in 10% acetonitrile, and lyophilized.

**Purification and Refolding of Peptides—**Each synthetic peptide was reduced by incubation for 2 h in 0.1 M Tris-HCl, pH 8.3, 0.1 M dithiothreitol (DTT), 1 mM EDTA. The solution was acidified to pH 3 with HCl and the reduced peptides purified by reverse phase HPLC (4) prior to refolding. The peptides were refolded using an oxido-shuffling system (15) for 20 h at room temperature in a solution containing 0.1 M Tris-HCl, pH 8.3, 50 mM CaCl2, 3 mM l-cysteine, 0.3 mM l-cystine at a concentration of <0.5 mg of peptide/ml. The reaction was stopped by adjusting to pH 3 and the products analyzed by reverse phase HPLC. The refolded wild-type peptide was purified by HPLC and lyophilized.

**Cloning and Expression of Mutant cbEGF Domain from Factor IX—**DNA encoding the cbEGF domain from human factor IX (residues 46–93) was amplified from genomic DNA isolated from a hemophilic patient (Oxford d2) with a mutation causing a G60S change (3, 14). This DNA fragment was cloned into pMB54 in frame with the yeast a-factor leader sequence and sequenced prior to transfer into pMA91. Transformation of yeast yeast and harvesting of secreted protein was as described previously (19). The protein containing fractions determined by SDS-polyacrylamide gel electrophoresis and immunoblotting were pooled, adjusted to 0.1 M DTT and 1 mM Tris-HCl, pH 8.3, reduced for 1–2 h at room temperature, and dialyzed against 0.1% (v/v) trifluoroacetic acid. The reduced proteins were purified by reverse phase HPLC (4), lyophilized, and refolded as described for the single domains. The refolded protein was lyophilized to concentrate the sample, digested with bovine factor Xa (DENZYME Aps, Aarhus, Denmark) in 50 mM Tris-HCl, pH 7.5, 0.1 mM NaCl, 5 mM CaCl2 at 37 °C for 20 h at a ratio of 1:1000 enzyme to protein to remove the 6XHis affinity tag, and the cleaved protein purified by reverse phase HPLC. The identity of the purified products was confirmed by amino acid analysis and mass spectroscopy.

**5,5'-Dithionitrobenzoic Acid (DTNB) Analysis—**Mutant peptides purified from the refolding mixture were assayed for free thiols with DTNB (20).

**ELISA of Patient Oxford d2—**Plasma from a hemophilia B patient Oxford d2 (14) with a G60S change was assayed for total FIX antigen using the monoclonal antibody H9 (21) and for its reactivity toward a monoclonal antibody 205, which recognizes a functionally significant epitope in human FIX located between amino acids 50 and 96 (12). ELISA assays were performed as described in Ref. 22. Pooled normal human plasma used as a standard was assumed to have a FIX concentration of 5 μg/ml.

**NMR Analysis—**All NMR spectra were acquired on home-built GE Omega spectrometers at 500 or 600 MHz. All samples used for titrations contained 150 mM NaCl to approximate physiological ionic strength (I = 0.15) and 5 mM Tris buffer unless otherwise specified. Calcium titrations were performed as described previously (19, 23). Calcium-dependent shifts of the mutant FIX cbEGF domain were assessed in a series of one-dimensional spectra recorded on a deuterated sample (≥200 μM) at pH 7.4 and 77 °C. The fibrillin cbEGF 13 titration was performed using a deuterated 714 μM sample at pH 6.5 and 77 °C. One-dimensional spectra were acquired with a spectral width of 8000 Hz and a block size of 4096 complex points. These data were zero-filled to 8192 points, yielding a digital resolution of 0.98 Hz/pixel. A series of two-dimensional NOESY spectra were acquired for measurement of calcium binding to the fibrillin cbEGF 13–14 pair, as described previously (19). 2048 complex points were acquired in F2 with a spectral width of 7017.54 Hz. These data were zero filled to 65k to yield a digital resolution of 0.86 Hz/pixel. The sample used in this titration was of 142 μM concentration, and the titration was performed at pH 6.5.
and \( T = 33 \, ^\circ \text{C} \). Measurement of the Tyr-1138 H\( ^6\)–He\( ^* \) cross-peak position was complicated by overlap of this peak at data points 2–4 with an unassigned cross-peak, and by overlap of this peak with the diagonal for the final points of the titration (see Fig. 8). An attempt was made to perform this titration on a deuterated sample using one-dimensional NMR; however, the H\( ^6\)–resonance was not well enough resolved to permit determination of the \( K_d \) from these data. Calcium-dependent shift of Tyr-1178 in the mutant fibrillin cbEGF domain pair were recorded on samples containing 1.77 mg of protein, respectively. The purified reduced peptides each gave a single peak by reverse phase HPLC, which was of the expected mass (Table I). Refolding of each single cbEGF domain resulted in a reduction of mass of 5–7 daltons consistent (within experimental error) with the formation of three disulfide bonds. Refolding of the wild-type form of fibrillin cbEGF13 produced an HPLC profile similar to that obtained previously for the wild-type form of the FIX cbEGF domain (4), in that the refolded material gave a single peak by reverse phase HPLC, which eluted earlier than the reduced form of the peptide (Fig. 4, A and B).

However, analysis of the refolded Gly \( \rightarrow \) Ser mutant forms of single cbEGF domains from fibrillin-1 and FIX showed markedly heterogeneous profiles (Fig. 4, C and D), despite the fact that refolded material collected from a cross-section of these chromatograms had the same mass (Table I). No free thiols (assayed by DTNB analysis, see “Experimental Procedures”) were detected in the single mutant domains. These data are consistent with the formation of misfolded monomeric peptide species. Two of the peaks identified in the chromatogram of refolded mutant fibrillin cbEGF13 were collected for NMR analysis (see Fig. 4D, arrows). All of the above refolding reactions were performed in the presence of Ca\(^{2+}\) (see “Experimental Procedures”), since this resulted in an increased yield of wild-type fibrillin cbEGF13 peptide with the native conformation (see below). In addition, Ca\(^{2+}\) enhanced the yield of one peak in the chromatogram of mutant FIX cbEGF domain (see Fig. 4C, arrow), which was subsequently analyzed using NMR.

In contrast to the results of single cbEGF domains, HPLC analysis of wild-type and mutant fibrillin cbEGF domain pairs showed no difference in elution profiles of wild-type versus mutant refolded peptide. Both refolded in the presence of Ca\(^{2+}\) to give one major species (data not shown). No free thiols were detected in the refolded mutant cbEGF pair.

ELISA of Oxford d2—Analysis of Oxford d2 plasma showed a total circulating FIX antigen of \(-1.3 \, \mu\text{g/ml} \) (mean of three experiments, individual values 1.27, 1.4, and 1.25 \( \mu\text{g/ml} \) measured by H9 ELISA. This corresponds to 26% of pooled normal plasma. This is in agreement with the antigen level (28%) previously recorded for this patient (14). Using 2D5, which recognizes a functionally significant epitope between residues 50–96 of human factor IX, 0.4 \( \mu\text{g/ml} \) (mean of three experiments; individual values 0.4, 0.42, and 0.37 \( \mu\text{g/ml} \) ) were detected. The 2D5/H9 ratio was \(-0.3\). This demonstrates that factor IX Oxford d2 has significant native-like conformation, although from these experiments we cannot distinguish be-
between 30% of mutant protein recognizing 2D5 with native affinity and 100% of mutant protein having a reduced affinity for 2D5.

NMR Structural Analysis of Single Domains and Domain Pairs—All wild-type constructs gave spectra consistent with folded species. In contrast to wild-type fibrillin cbEGF 13, one-dimensional spectra acquired for HPLC peaks 1 and 2 of the mutant form of fibrillin cbEGF 13 (see Fig. 4D) were broad with poor dispersion of amide resonances, no upfield shifted methyl resonances, and no downfield shifted C=H resonances (which are characteristic of β-sheet secondary structure). The data are consistent with a heterogeneous sample, with no major component containing native-like structure. It is possible, however, that one or both of these samples contained a minor fraction of correctly folded material that was not of sufficient concentration to be observable in the spectra.

A comparison of two-dimensional NOESY spectra of the wild-type and mutant containing fibrillin cbEGF 13–14 pairs is shown in Fig. 5. Superposition of these spectra reveals that a subset of the peaks in the spectrum of the wild-type pair is present in the spectrum of the mutant. The position of the Tyr-1178 H8–H9 resonance is virtually identical in the two spectra, while the cross-peak corresponding to Tyr-1136 is absent in the spectrum of the mutant. These data indicate that domain 14 is correctly folded, and domain 13 is not. Correct folding of domain 14 of the mutant cbEGF pair was confirmed by observation of calcium-dependent chemical shift of the Tyr-1178 cross-peak (see below). The absence of peaks corresponding to domain 13 in the mutant cbEGF pair spectrum implies that a range of misfolded conformers of this domain exist in solution. The absence of free thiols in the sample of the mutant pair and mass spectrometric analysis show that misfolding is confined to domain 13, and no higher molecular weight disulfide cross-linked products were present in the NMR sample.

Calcium Binding of cbEGF Domains—To confirm correct folding of a proportion of refolded mutant FIX cbEGF domain and of domain 14 in the mutant fibrillin cbEGF pair, calcium-dependent shifts were observed in one- and two-dimensional spectra, respectively. Analysis of the well resolved Ca2+-dependent peak present in the chromatogram of mutant FIX cbEGF domain (Fig. 4, arrow) by one-dimensional NMR identified features of a folded protein, including Ca2+-dependent chemical shifts in the aromatic region of the spectrum (Fig. 6D). This suggests that the mutant domain can adopt a calcium-dependent conformation, similar to the wild-type domain (3, 16). No calcium-dependent chemical shifts were identified in spectra recorded for the other HPLC peaks derived from refolding mutant FIX cbEGF domain (Fig. 6, A–C). In addition, no calcium-dependent shifts were observed for HPLC peaks 1 and 2 of mutant fibrillin cbEGF 13, in agreement with the conclusion that neither of these samples contained a significant fraction of material with the wild-type fold.

Chemical shift of conserved aromatic residues as a function of calcium concentration were used to derive dissociation constants for wild-type fibrillin cbEGF 13 and the cbEGF 13–14 pair (Fig. 7). Both values are consistent with those measured in similar fibrillin cbEGF domain constructs (19, 23). Wild-type fibrillin cbEGF 13 had a dissociation constant of ~2–3 mM. The

**Table I**

Characterization of purified cbEGF domain constructs

| Peptide         | Reduced mass | Oxidized mass | Method of production |
|-----------------|-------------|--------------|---------------------|
| FIX G60S        | 4307.3      | 4300.88, 4301.55 | Yeast              |
|                 | (4307.7)    | (4301.56)    |                     |
| FIB cbEGF13     | 4719.43     | 4714.25      | Peptide synthesis   |
|                 | (4719.37)   | (4713.32)    |                     |
| FIB cbEGF13     | 4749.38     | 4742.5, 4742.75 | Peptide synthesis   |
|                 | (4749.4)    | (4743.35)    |                     |
| G1127S          | 5330.22     | 5325.34      | E. coli            |
|                 | (5330.22)   | (5325.34)    |                     |
| FIB cbEGF13-14  | 11,367.22   | 9526.36      | E. coli            |
|                 | (11,368.87) | (9526.76)    |                     |
| FIB cbEGF13-14  | 11,398.93   | 9532.74      | E. coli            |
|                 | (11,398.89) | (9532.79)    |                     |

*a* Mass obtained includes the 6xHis tag and FXa cleavage site which is only cleaved off after the peptide has been oxidized.

**Fig. 4.** A and B, HPLC chromatograms illustrating the elution profile of wild-type fibrillin cbEGF 13 on reduction with DTT (A) and on refolding using an oxidized-shuffling system (B). In C, the heterogeneous profile of refolded mutant FIX cbEGF domain is shown, and the peak that increases in intensity when refolding is conducted in the presence of calcium is highlighted by an arrow. In D, the heterogeneous profile of refolded mutant fibrillin cbEGF 13 is shown, and the two HPLC peaks selected for NMR analysis are highlighted by arrows.
affinity of the calcium binding site in the C-terminal domain \((\leq 100 \mu M)\) of the cbEGF 13–14 pair measured at least an order of magnitude higher than the site in the N-terminal domain \((-3 \text{mM})\). Fig. 8 shows the aromatic region of the spectrum of the cbEGF 13–14 pair at zero and at saturating calcium concentrations. The \(K_d\) of domain 14 is at the limit of the range of calcium dissociation constants that can be measured by NMR. Domain 14 of the mutant fibrillin cbEGF 13–14 pair showed calcium-dependent changes in chemical shift consistent with this domain having wild-type calcium affinity (data not shown), despite the fact that the adjacent domain was misfolded.

**DISCUSSION**

Our previous studies on cbEGF domains have demonstrated their ability to fold \textit{in vitro} (either as single or pairs of domains) into their native conformation. We have utilized \(^{1}H\) NMR techniques to assay characteristic structural features of the cbEGF domain, which include \(\beta\)-sheet formation and calcium binding. In this study, we analyzed the effects of a Gly \(\rightarrow\) Ser amino acid change that, because of its strategic position in the cbEGF domain (see Fig. 1), was predicted to cause misfolding. HPLC and NMR data for the wild-type and mutant cbEGF domain constructs from fibrillin and factor IX demonstrated that this type of amino acid change is associated with a variable degree of misfolding \textit{in vitro}, which is dependent on the exact sequence of the cbEGF domain. It is difficult to determine accurately the fraction of native-like fold associated with each of the intact proteins \textit{in vivo}, particularly since fibrillin, a structural component of extracellular microfibrils, is not easily assayed. However, we can consider the structural basis for the misfolding. When the Gly \(\rightarrow\) Ser mutation is introduced by molecular modelling into either the FIX or the fibrillin wild-type domain, a steric clash is observed between the serine side chain and the consensus disulfide bond connecting cysteines 5 and 6. Hence, one could speculate that a Gly \(\rightarrow\) Ser change could interfere with the folding pathway associated with formation of this disulfide bond.

Despite there being a clear structural basis for misfolding when the Gly \(\rightarrow\) Ser amino acid change is present in cbEGF domains, this change is associated with relatively mild disease. In FIX this mutation has been identified in over 50 hemophiliac
Defective Folding of cbEGF Domains

Saturating concentration of calcium (>250 mM).

Calcium dissociation constants for each cbEGF domain were derived as described previously (19, 23). Standard regression analysis was used to curve fit the data for each site using the equation \[ \Delta = \Delta_0 [Ca^{2+}]_{free}/K_d + [Ca^{2+}]_{free}. \]

Note that the path of the Tyr-1136 peak intersects an unassigned peak, which manifests a much smaller total chemical shift migration, and that the displacement of the Tyr-1136 and Tyr-1178 cross-peaks is indicated. The change in chemical shift of the Hα resonances of tyrosine residues 1136 and 1178 in the isolated fibrillin cbEGF 13 (●) and in the cbEGF 13–14 pair (●, ○) as a function of free calcium concentration. Calcium dissociation constants for each cbEGF domain were derived as described previously (19, 23).

The possible consequences of these results for expression of the phenotype associated with the G1127S change are (i) that, although not detected in vitro, a proportion of correctly folded cbEGF domain harboring the mutation is formed in vivo (its formation facilitated by sequential and cotranslational folding in eukaryotic cells and the presence of chaperones) and is secreted by the cell. It is then incorporated into the microfibril, together with wild-type protein where it produces a relatively mild effect, (ii) since the effect of the G1127S change on folding appears to be “self-contained” within a cbEGF domain, and does not interfere with the folding or calcium binding properties of the adjacent cbEGF domain in vitro, a proportion of the misfolded material may be secreted from the cell and assembled into the microfibril. These two possibilities (which are not mutually exclusive) are consistent with the analysis of a fibroblast cell line obtained from a patient with the G1127S change, which showed normal synthesis of total fibrillin, but reduced deposition in the matrix (13).

Acknowledgments—We thank Dr. H. Reisner for the 2D5 antibody. We thank Dr. Robin Aplin for mass spectrometry analyses and Dr. Maureen Pitkeathly for peptide synthesis. We acknowledge the assistance of J. Marriott in preparation of figures.

REFERENCES
1. Campbell, I. D., and Bork, P. (1993) Curr. Opin. Struct. Biol. 3, 385–392
2. Rees, D. J. G., Jones, I. M., Handford, P. A., Walter, S. J., Esonouf, M. P., Smith, K. J., and Brownlee, G. G. (1988) EMBO J. 7, 2053–2061
3. Handford, P. A., Mayhew, M., Baron, M., Winship, P. R., Campbell, I. D., and Brownlee, G. G. (1991) Nature 351, 164–167
4. Mayhew, M., Handford, P., Baron, M., Tse, A. G., Campbell, I. D., and Brownlee, G. G. (1992) Proteins 18, 489–494
5. Rao, Z., Handford, P., Mayhew, M., Knott, V., Brownlee, G. G., and Stuart, D. (1995) Cell 82, 131–141
6. Downing, A. K., Knott, V., Werner, J. M., Cardy, C. M., Campbell, I. D., and Handford, P. A. (1996) Cell 85, 597–605
7. Giannelli, F., Green, P. M., Sommer, S. S., Poon, M., Ludwig, M., Schwaab, R., Reitsma, P. H., Gossens, M., Yoshioka, A., and Brownlee, G. G. (1996) Nucleic Acids Res. 24, 103–118
8. Collod-Béroud, G., Béroud, C., Ades, L., Black, C., Boxer, M., Brock, D. J., Godfrey, M., Hayward, C., Karttunen, L., Milewicz, D., Peltonen, L., Rich-ards, R. I., Wang, M., Junien, C., and Boileau, C. (1997) Nucleic Acids Res. 25, 147–150
9. Joutel, A., Corpechot, C., Ducros, A., Vahedi, K., Chabrier, H., Mouton, P., Alamowitch, S., Demenga, V., Cecilion, M., Marechal, E., Maciazek, J.,
Defective Folding of cbEGF Domains

Vayssière, C., Craaud, C., Cabanis, E., Ruchoux, M., Weissbach, J., Bach, J., Bossier, M., and Tournier-Lasserve, E. (1996) Nature 338, 707–710
10. Hobbs, H. H., Brown, M. S., and Goldstein, J. L. (1992) Hum. Mol. Genet. 1, 445–466
11. Gandrille, S., Borgel, D., Eschwege-Gufflet, V., Aillaud, M., Dreyfus, M., Matheron, C., Gaussem, P., Abgrall, J. F., Jude, B., Sie, P., Toulon, P., and Aisch, M. (1995) Blood 85, 130–138
12. Denton, P. H., Fowlkes, D. M., Lord, S. L., and Reisner, H. M. (1988) Blood 72, 1407–1411
13. Franke, U., Berg, M. A., Tynan, K., Brenz, T., Liu, W., Aoyama, T., Gasner, C., Miller, D. C., and Furtihrnay, H. (1995) Am. J. Hum. Genet. 56, 1287–1296
14. Winship, P. R., and Dragon, A. C. (1991) Br. J. Haematol. 77, 102–109
15. Jaenicke, R., and Rudolph, R. (1989) in Protein Structure: A Practical Approach (Creighton, T. E., ed) pp. 208–209, IRL Press, Oxford
16. Handford, P. A., Baron, M., Mayhew, M., Willis, A., Beesley, T., Brownlee, G. G., and Campbell, I. D. (1990) EMBO J. 9, 475–480
17. Pereira, L., D’Alessio, M., Ramirez, F., Lynch, J. R., Sykes, B., Pangilinan, T., and Bonadio, J. (1993) Hum. Mol. Genet. 2, 961–968
18. Raleigh, E. A., Murray, N. E., Revel, H., Blumenthal, R. M., Westaway, D., Reith, A. D., Rigby, P. W. J., Rigby, Elhai, J., and Hanahan, D. (1988) Nucleic Acids Res. 16, 1563–1575
19. Knott, V., Downing, A. K., Cardy, C. M., and Handford, P. (1996) J. Mol. Biol. 255, 22–27
20. Ellman, G. L. (1977) Arch. Biochem. Biophys. 82, 70–77
21. Yoshiooka, A. Giddings, J. C., Thomas, J. E., Fujimura, Y., and Bloom, A. R. (1985) Br. J. Haematol. 59, 265–275
22. Handford, P. A., Winship, P. R., and Brownlee, G. G. (1991) Protein Eng. 4, 319–333
23. Handford, P., Downing, A. K., Rao, Z., Hewett, D. R., Sykes, B. C., and Kielty, C. M. (1995) J. Biol. Chem. 270, 6751–6756
24. Ketterling, R. P., Bottema, C. D. K., Phillips, J. A., and Sommer, S. S. (1991) Genomics 10, 1093–1096
25. Sakai, L. Y., Keene, D. R., and Engel, E. (1986) J. Cell Biol. 103, 2499–2509
26. Sakai, L. Y., Keene, D. R., Glavette, R. W., and Bachinger, H. P. (1991) J. Biol. Chem. 266, 14763–14770
27. Dietz, H. C., and Pyeritz, R. E. (1995) Hum. Mol. Genet. 4, 1799–1809
A Gly → Ser Change Causes Defective Folding in Vitro of Calcium-binding Epidermal Growth Factor-like Domains from Factor IX and Fibrillin-1
Pat Whiteman, A. Kristina Downing, Rachel Smallridge, Peter R. Winship and Penny A. Handford

*J. Biol. Chem.* 1998, 273:7807-7813. doi: 10.1074/jbc.273.14.7807

Access the most updated version of this article at http://www.jbc.org/content/273/14/7807

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 26 references, 5 of which can be accessed free at http://www.jbc.org/content/273/14/7807.full.html#ref-list-1