Biophysical Characterization of the Signature Domains of Thrombospondin-4 and Thrombospondin-2*

Tina M. Misenheimer1 and Deane F. Mosher
From the Department of Medicine, University of Wisconsin-Madison, Madison, Wisconsin 53706

The signature domain of thrombospondins consists of tandem epidermal growth factor-like modules, 13 calcium-binding repeats, and a lectin-like module. Although very similar, the signature domains of thrombospondin-1 and -2 differ in several potentially important ways from the domains of thrombospondin-3, -4, and -5. We have compared matching recombinant segments representing the signature domains of thrombospondin-2 and -4. In the presence of 2 mM CaCl₂, the far UV circular dichroism spectra of thrombospondin-2 and -4 constructs contain a strong negative band at 202 nm, but only the thrombospondin-2 construct has a band at 216 nm. Chelation of calcium shifted the negative bands to lower magnitudes. Titrations of the spectra demonstrated lower cooperativity and affinity for binding of calcium to thrombospondin-4 compared with thrombospondin-2. Atomic absorption spectroscopy demonstrated that the thrombospondin-4 constructs bind seven less calcium than the thrombospondin-2 construct at 0.6 mM CaCl₂. In 2 mM CaCl₂, the near UV circular dichroism spectra of thrombospondin-2, but not thrombospondin-4, contain a positive band at 292 nm that disappears upon calcium chelation. Intrinsic fluorescence spectra for both proteins were also sensitive to calcium, but the changes were similar and more marked for thrombospondin-2 than for thrombospondin-4. In differential scanning calorimetry, the thrombospondin-2 construct melted in two distinct transitions at 53.5 and 81.8 °C, whereas the first transition for thrombospondin-4 constructs was observed at 63.5 °C. Thus, the studies revealed significant differences between the signature domains of thrombospondin-2 and thrombospondin-4 in calcium binding, fine structure, and inter-modular interactions.

* This work was supported by National Institutes of Health Grant HL54462. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed: Dept. of Medicine, University of Wisconsin-Madison, 1300 University Ave., Madison, WI 53706. Tel.: 608-262-3189; Fax: 608-263-4969; E-mail: tmmisenh@wisc.edu.

2 The abbreviations used are: TSP, thrombospondin; CD, circular dichroism; E, epidermal growth factor-like module of thrombospondin; EGF, epidermal growth factor; E122CaG-4, residues 286–961 of human thrombospondin-4; E123CaG-2, residues 551–1172 of human thrombospondin-2; G, C-terminal globule of thrombospondin; λmax, wavelength of maximum fluorescence; DSC, differential scanning calorimetry; MOPS, 4-morpholinepropanesulfonic acid.
of these comparisons, we tested TSP-4 constructs that harbored either the Ala387 or Pro387 allele in the E2’ module.

MATERIALS AND METHODS

Cloning of E122’3CaG-4 Ala387 and E122’3CaG-4 Pro387 — The pAcGP67.coco baculovirus transfer vector, which contains DNA encoding a signal peptide 5’ to and a six-histidine tag (His tag) 3’ to the cloning site, was used to facilitate baculovirus-mediated protein expression (18). E122’3CaG-4 Ala387 (residues 286–961) was PCR-amplified from a full-length clone of human TSP-4 (a generous donation of Drs. Jack Lawler, Harvard Medical School, Boston, MA; and Faye Hui Chen, Columbia University, New York, NY), and inserted into pAcGP67.coco. The TSP-4 clone has a Val at position 737 (as indicated in Swiss-Prot entry P35443) instead of the Gly737 originally published for human TSP-4 (19). Swiss-Prot describes rat, mouse, and *Xenopus* TSP-4 as

![Figure 1. Schematic diagram of TSP constructs.](image1)

The N-terminal heparin-binding module (N), oligomerization domain (O), procollagen module (C), properdin modules (P), EGF-like modules (E), C-terminal sequence (G) are indicated in the diagram. The TSP-4 monomer lacks the procollagen and properdin modules and contains a fourth EGF-like module. An arrow marks the location of Ala387 in TSP-4 that is changed to Pro387 in the polymorphism (14). The constructs described in the paper are named according to the modules present and from which TSP form the modules are derived.

![Figure 2. Sequence alignment of hTSP-4 and hTSP-2.](image2)

The amino acid sequences of the C-terminal regions of hTSP-4 and hTSP-2 are aligned and numbered starting with the initiating methionine. Identical residues are indicated with an * underneath the sequences. The modules are labeled according to TSP-2 nomenclature: EGF-like modules E1, E2, etc.; calcium-binding domain subdivided into its 13 repeats of either C-type or N-type calcium binding (4, 6); and the C-terminal globe, G. The areas where TSP-4 and TSP-2 differ are coded: the extra EGF in TSP-4 (E2’/H11032) is bold, Ala387 is highlighted gray, the site of N-glycosylation in the calcium-binding repeat 1C of TSP-2 that is deleted in TSP-4 is bold, Asp744 and Asn746 in calcium-binding repeat 3C of TSP-2 are bold, the four-residue insertion in the calcium-binding repeat 11C of TSP-4 is bold, the extra residues in the C-terminal tail of TSP-4 are bold, and the extra cysteine (Cys925) in the C-terminal globe of TSP-4 is bold and italicized. The calcium binding and \(\beta\) hydroxylation consensus sequences in the EGF-like modules are underlined (17). The tryptophan residues are highlighted gray.
having Val at the equivalent position. The Pro<sup>387</sup> polymorphism was introduced by PCR mutagenesis into E122<sup>3</sup>CaG-4. Correct orientation and sequence of PCR-amplified DNA were verified by automated sequencing.

**Expression and Purification of Recombinant Proteins**—The proteins were expressed by infecting High Five insect cells in SP900II serum-free medium at 22 °C with high titer virus (>10<sup>7</sup> plaque-forming units/ml) at a multiplicity of infection of 5. Conditioned medium was collected ~65 h post-infection. Histidine-tagged proteins were purified from the medium in the presence of 2 mM CaCl<sub>2</sub> using Ni<sup>2+</sup>-nitrilotriacetic acid resin (Qiagen) as described previously (4, 7, 18). Protein concentration was determined using absorbance at 280 nm and calculated extinction coefficients of 1.07 and 1.24 ml mg<sup>-1</sup> cm<sup>-1</sup> for E122<sup>3</sup>CaG-4 and E123CaG-2, respectively (20).

**Calcium Binding**—The ability of purified proteins to bind Ca<sup>2+</sup> was measured directly using atomic absorption spectroscopy (7). Atomic absorption quantification of calcium content was done at the University of Wisconsin Chemistry Department using a Solaar Unicam 969 flame atomic absorption spectrometer. Prior to analysis, the proteins were dialyzed into 10 mM MOPS, 0.15 M NaCl, pH 7.5 containing 0.6 mM CaCl<sub>2</sub>. Protein solution and dialysis buffer were analyzed for calcium using calcium carbonate as the standard. Atomic absorption values are expressed as the mean ± S.E. of 3–4 experiments.

**Circular Dichroism**—CD studies were done at the University of Wisconsin Biophysics Instrumentation Facility using an Aviv 62A DS CD spectrometer. Far UV light measurements were taken at 37 °C in a 0.1–cm path length cuvette using protein (0.08–0.10 mg/ml) dialyzed into 5 mM MOPS, 100 mM NaCl, 2 mM CaCl<sub>2</sub>, pH 7.5. Calcium titrations were analyzed as described previously (7) by comparing the fractional change (R) in mean residue ellipticity at 210 nm at given calcium concentrations divided by the difference in values observed when no further changes occur upon the addition of EDTA. The Hill equation that describes this plot is log(R/1−R) = n log[Ca<sup>2+</sup>] + log K, where n is the Hill coefficient and K is an equilibrium dissociation constant (21). Near UV light measurements were taken at 25 °C in a 1 cm path length cuvette using protein (0.8–1.2 mg/ml) dialyzed into 10 mM MOPS, 150 mM NaCl, 2 mM CaCl<sub>2</sub>, pH 7.5.

**Differential Scanning Calorimetry**—DSC was done at the University of Wisconsin Biophysics Instrumentation Facility using a Microcal differential scanning calorimeter with proteins (0.8–1.5 mg/ml) dialyzed into 10 mM MOPS, 150 mM NaCl, 2 mM CaCl<sub>2</sub>, pH 7.5. Scans were from 15 to 95 °C at a rate of 60 °C/h.

**Intrinsic Fluorescence**—Fluorescence emission spectra of purified proteins (0.04–0.05 mg/ml) in 5 mM MOPS, 100 mM NaCl, 2 mM CaCl<sub>2</sub>, pH 7.5 were obtained at 37 °C using a JY Fluoromax-3 fluorometer with excitation at 295 nm. Fluorescence spectra were obtained for each protein after each addition of EDTA. Calcium titrations were analyzed by calculating the relative fluorescence intensities at 332 nm at each calcium concentration (fluorescence intensity at a given calcium concentration divided by the fluorescence intensity at 2 mM CaCl<sub>2</sub>). Fluorescence was also monitored during thermal denaturation and upon

**FIGURE 3.** Far UV CD spectra. Far UV CD spectra for E123CaG-2 (dashed line), E122<sup>3</sup>CaG-4 Ala<sup>387</sup> (solid line), and E122<sup>3</sup>CaG-4 Pro<sup>387</sup> (dotted line) are shown in the presence of 2 mM CaCl<sub>2</sub> (A) and upon removal of CaCl<sub>2</sub> by the addition of 2 mM EDTA (B). Spectra were measured at 37 °C in 5 mM MOPS, 100 mM NaCl, 0–2 mM CaCl<sub>2</sub>, pH 7.5. Representative spectra are shown.

**FIGURE 4.** Far UV CD titration curves. Far UV CD spectra for E123CaG-2 (open circles), E122<sup>3</sup>CaG-4 Ala<sup>387</sup> (closed triangles), and E122<sup>3</sup>CaG-4 Pro<sup>387</sup> (open triangles) were measured at 37 °C in 5 mM MOPS, 100 mM NaCl, 0–2 mM CaCl<sub>2</sub>, pH 7.5. Protein, initially in 2 mM CaCl<sub>2</sub>, was treated with increasing amounts of EDTA to remove CaCl<sub>2</sub>. Results were analyzed by comparing the fractional change in molar ellipticity at 210 nm at a given calcium concentration divided by the difference in values observed when no further changes occur upon the addition of EDTA (7). Values are expressed as the mean ± S.E. of four experiments.
RESULTS

For comparisons of the TSP-4 signature domain to the E123CaG-2 signature domain of TSP-2, for which there is a crystal structure (4), we expressed E122’3CaG-4 containing either Ala or Pro at position 387 in E2’ (Fig. 1). When the sequences of TSP-2 and -4 are aligned, it is apparent that E2 of TSP-4 is most like E2 of TSP-2, and the extra EGF-like module (E2’), although most like E2, is the EGF-like module that is present in TSP-4 and missing in TSP-2 (Fig. 2). The proteins were cloned and expressed using a baculovirus expression system and purified using nickel chelate chromatography (7, 18). The yields of purified TSP-4 proteins were 10–40 mg/liter conditioned medium. This level of expression is comparable with the expression of the TSP-2 proteins (7) and much higher than the secretion of intact TSP-1 or -2 under the same conditions (data not shown). All truncated proteins were soluble and amenable to structural and functional analyses.

Far UV Circular Dichroism—In the presence of calcium, both E122’3CaG-4 constructs and E123CaG-2 have a negative band at 202 nm (Fig. 3). The E122’3CaG-4 spectra, however, lack a negative band at 216 nm that is apparent in E123CaG-2 (Fig. 3A), as well as full-length TSP-1 (21) and E3CaG-2 (7). Calcium chelation shifts the spectra of all three proteins to lower magnitudes (Fig. 3B and 3C). For comparisons of the TSP-2 proteins (7) and much higher than the secretion of intact TSP-1 or -2 under the same conditions (data not shown). All truncated proteins were soluble and amenable to structural and functional analyses.

Calcium Binding—Calcium binding of the constructs in buffer containing 0.6 mM CaCl₂ was measured using atomic absorption spectroscopy. Because the analysis requires subtraction of the calcium content in dialysis buffer from the calcium content of the protein solution, this was the highest calcium concentration at which there was enough enrichment in the protein solutions to obtain an accurate measurement. E123CaG-2 bound 28 ± 1 Ca²⁺/protein. This result is consistent with what we reported previously for E3CaG-2 (7) and in agreement with the 30 bound calcium ions found in the crystal structure of E123CaG-2 (4). The TSP-4 constructs bound seven less calcium ions than the TSP-2 construct, 21 ± 2 Ca²⁺/protein for E122’3CaG-4 Ala²⁺ and 20 ± 1 Ca²⁺/protein for E122’3CaG-4 Pro²⁺.

Near UV CD. Near UV CD spectra for E123CaG-2 (dashed line), E122’3CaG-4 Ala²⁺ (solid line), and E122’3CaG-4 Pro²⁺ (dotted line) are shown in the presence of 2 mM CaCl₂ (A) and upon removal of CaCl₂ by the addition of 2 mM EDTA (B). Spectra were measured at 25 °C in 10 mM MOPS, 150 mM NaCl, 0–2 mM CaCl₂, pH 7.5. Representative spectra are shown.

Differential Scanning Calorimetry—Thermal stability of the proteins was measured using DSC. In 2 mM CaCl₂, E123CaG-2 melted with two transitions (53.5 and 81.8 °C) (Fig. 5), similar to the reported transitions for E3CaG-2 (50.5 and 82.7 °C) (7). Under identical conditions, we were only able to measure one transition for E122’3CaG-4 Ala²⁺ and Pro²⁺ at 63.3 and 63.5 °C, respectively (Fig. 5). We do not know whether a higher transition is present, because both E123CaG-2 and the E122’3CaG-4 constructs precipitated between 75 and 80 °C, and the experiments had to be discontinued.

Fluorescence Spectroscopy—Intrinsic fluorescence of tryptophan residues is sensitive to perturbations in the local protein structure. The intrinsic fluorescence of the seven conserved tryptophans has been shown to be calcium-sensitive and to dominate the fluorescence spectra of E3CaG-2 (7). Because G has been found to bind three calcium ions in TSP-2 (4) and 4 calcium ions in TSP-1 (6) and to interact intimately with the calcium binding repeats of thrombospondin and E3, the tryptophan fluorescence could be influenced by removal of calcium from G or disruption of interactions of G with the calcium binding repeats of thrombospondin and the EGF-like repeats.
The local tryptophan environment was examined by measuring intrinsic fluorescence after excitation at 295 nm (Fig. 7). The fluorescence emission spectra for all three proteins are sensitive to the presence of calcium. In the presence of calcium, the wavelength of maximum fluorescence ($\lambda_{\text{max}}$) is about 332 nm for the three proteins, but the shift in $\lambda_{\text{max}}$ upon removal of calcium is more for E123CaG-2 than for E122'3CaG-4 Ala387 or E122'3CaG-4 Pro387. The $\lambda_{\text{max}}$ for E123CaG-2 increased 4.5 nm from 332.5 nm at 2 mM CaCl$_2$ to 337 nm at 0 mM CaCl$_2$, whereas the $\lambda_{\text{max}}$ for the E122'3CaG-4 proteins only increased 1.5 nm from 332 to 333.5 nm (TABLE ONE). Calcium sensitivity of the intrinsic fluorescence was examined in more detail by titration of calcium-replete E122'3CaG-4 Ala387, E122'3CaG-4 Pro387, and E123CaG-2 with increasing amounts of EDTA (Fig. 8). The fluorescence intensity of E122'3CaG-4 Ala or Pro387 initially decreased gradually as calcium was removed and then jumped back to the starting intensity with an EC$_{50}$ value of 560 mM for the second transition. The fluorescence intensity of E123CaG-2, however, continually decreased as calcium was removed, with a resulting EC$_{50}$ value of 980 mM calcium.

An increase in $\lambda_{\text{max}}$ indicates the local area around the tryptophan residues unfolds, resulting in an increased exposure of the tryptophan residues to water. Intrinsic fluorescence was also monitored during thermal denaturation and titration with guanidine hydrochloride. In all cases, the degree of unfolding observed, as measured by change in $\lambda_{\text{max}}$, was less upon removal of calcium by EDTA than upon thermal denaturation or denaturation with 5 M guanidine hydrochloride (TABLE ONE). The $\lambda_{\text{max}}$ for all three proteins at 37 °C in 2 mM CaCl$_2$ was ~332 nm and shifted to 338–339 nm upon heating to 70 °C or to 348 nm upon the addition of 5 M guanidine hydrochloride, suggesting that calcium depletion does not denature either protein, but rather, shifts the proteins to new folded states.
DISCUSSION

Comparison of the TSP-4 E122‘3CaG-4 constructs to the analogous TSP-2 construct E123CaG-2 using CD, fluorescence, atomic absorption, and calorimetry reveal significant differences in structure and calcium-binding properties between the signature domains of the two TSPs. Because the signature domain of TSP-4 is most similar to that of TSP-3 and next most similar to TSP-5 (3), the analyses of TSP-4 structure are pertinent to understanding all three of the pentameric TSPs.

Atomic absorption spectroscopy showed that, in 0.6 mM CaCl$_2$, E122‘3CaG-4 binds seven less calcium ions than E123CaG-2. The 28 ± 1 calcium ions bound to E123CaG-2 using atomic absorption spectroscopy agrees with the crystal structure of E123CaG-2. The far UV CD titration data indicate that the structural change in E123CaG-2 upon calcium binding is complete by 0.5 mM CaCl$_2$, but only 40–50% complete for E122‘3CaG-4 (Fig. 4). Therefore, complete binding would not be expected for the TSP-4 constructs at 0.6 mM CaCl$_2$, in which case E122‘3CaG-4 may lack fewer than 7 calcium-binding sites when fully saturated. Asp$^{744}$ and Asn$^{746}$ of repeat 3C of TSP-2 are replaced with Ala/His at 37 °C in 5 mM MOPS, 100 mM NaCl, 0–2 mM CaCl$_2$ (7). Values are expressed as the mean ± S.E. of four experiments.

**TABLE ONE**

| Protein                | 2 mM Ca$^{2+}$ | 0 mM Ca$^{2+}$ | 2 mM Ca$^{2+}$ | 5 M GuHCl 2 mM Ca$^{2+}$ |
|------------------------|----------------|----------------|----------------|--------------------------|
|                        | 37 °C          | 37 °C          | 70 °C          | 37 °C                    |
| E123CaG-2              | 332.5          | 337            | 339            | 348                      |
| E122‘3CaG-4 Ala$^{387}$| 332            | 333.5          | 339            | 348                      |
| E122‘3CaG-4 Pro$^{387}$| 331.5          | 333.5          | 338            | 348                      |

**FIGURE 8. Titration of intrinsic fluorescence.**

Intrinsic fluorescence spectra of E123CaG-2 (open circles), E122‘3CaG-4 Ala$^{387}$ (closed triangles), and E122‘3CaG-4 Pro$^{387}$ (open triangles) were measured at 37 °C in 5 mM MOPS, 100 mM NaCl, 0–2 mM CaCl$_2$, pH 7.5, after excitation at 295 nm. Titration curves were made by the addition of increasing amounts of EDTA. Results at different calcium concentrations are compared by calculating the fluorescence intensity relative to that observed at 2 mM CaCl$_2$ (7). Values are expressed as the mean ± S.E. of four experiments.
amax value of ~332 nm (Fig. 7), removal of calcium red shifts the amax value of 4.5 nm for E123CaG-2 and only 1.5 nm for E122’3CaG-4 (TABLE ONE), and calcium titration curves monitored using fluorescence have two transitions for TSP-4 and only one for TSP-2 (Fig. 8).

DSC revealed differences in the thermal stability of the TSP-4 and -2 proteins. E123CaG-2 melts with transitions at 53.5° and 81.8 °C (Fig. 5). Previously, we found that E3CaG-2 melts with transitions at 50.5° and 82.7 °C (7). The first transition was assigned to unfolding of the intermodular interface among E3, the calcium-binding repeats, and G, whereas the second transition was assigned to the unfolding of the calcium-binding repeats (7). The increase in the first transition, when E12 is added to E3CaG-2, is compatible with the observed interaction between the C-terminal part of the calcium-binding repeats and E2 in the crystal structure of E123CaG-2 (4). Only one transition at 63.5 °C was observed for the TSP-4 proteins before they precipitated (Fig. 5). Because the proteins precipitated between 75° and 80 °C, it is unknown whether another transition would have occurred if the experiment could have been completed. In other words, the 63.5° transition may be the only transition, thereby corresponding to both the unfolding of the calcium-binding repeats and the unfolding of the intermodular interface, or it may be the first transition corresponding to the unfolding of the interface only. Either way, a difference in stability is apparent between the E122’3CaG-4 constructs and the homologous E123CaG-2 construct.

Without a crystal structure for TSP-4, however, the exact nature of the difference is unknown.

Because of the putative role of the A387P TSP-4 polymorphism in premature myocardial infarction (14–16), we expressed the C-terminal region of hTSP-4 as both the common (Ala387) and polymorphic (Pro387) forms. High levels of TSP-4 mRNA have been detected in heart and skeletal muscles (9), and human endothelial cells and vascular smooth muscle cells from brain blood vessels and coronary arteries have been shown to express TSP-4 mRNA (10). A recombinant E122’3CaG piece of TSP-4 with the Pro387 allele suppresses endothelial cell adhesion and proliferation, suggesting that it is a “gain of function” polymorphism that is proatherogenic (10). Recently, an E122’3Pro387 construct has been reported to have an additional Tb3+-binding site (27). However, our analyses failed to reveal significant structural differences in E122’3CaG-4 due to the polymorphism. Atomic absorption spectroscopy indicated that both TSP-4 proteins bind the same number of calcium ions. Far UV CD, near UV CD, and intrinsic fluorescence spectra for E122’3CaG-4 Ala387 and Pro387 are virtually identical (Figs. 3, 4, 6, and 7), and DSC showed similar thermal stabilities (Fig. 5). Therefore, the A387P single nucleotide polymorphism does not appear to affect the overall protein structure of E122’3CaG-4 nor does it alter its calcium-binding properties. The polymorphic TSP-4 contains a Pro instead of an Ala at position 387 in the third EGF-like module (E2) of TSP-4 (Fig. 2).

Comparative NMR analysis of a probe to alanine (P1148A) missense mutation in calcium binding EGF13 of fibrillin-1 has revealed that the fibrillin mutation also does not result in a structural defect in EGF13 or the adjacent EGFs (28). Similar to Pro1148 of fibrillin-1, Pro387 of TSP-4 is in a calcium-binding EGF and is expected to be in a turn (not a β-sheet) (4). The striking differences in the effects of the constructs based on common and polymorphic TSP-4 on the phenotype of endothelial cells (10) is, therefore, likely due to an altered interaction with some cellular component caused by a local structural change in the third EGF rather than by an overall structural change.

Together, the calcium binding, spectroscopic, and calorimetric data reveal significant structural differences in the C-terminal regions of TSP-2 and -4, especially in the lectin-like G domain. Further analysis of TSP-4 is necessary to determine which structural differences account for the altered calcium-binding properties. It may not be any single difference but rather a combination of differences that leads to fewer calcium-binding sites in TSP-4, a decrease in cooperativity of binding, and differences in fluorescence and CD spectra.

Acknowledgments—We acknowledge the University of Wisconsin-Madison Biophysics Instrumentation Facility, which is supported by the University of Wisconsin-Madison and Grants BIR-9512577 (National Science Foundation) and S10 RR135790 (NIH).

REFERENCES
1. Adams, J. C., Tucker R. P., Lawler, J. (1995) The Thrombospondin Gene Family, Molecular Biology Intelligence Unit, R. G. Landes Company, Austin, TX
2. Adams, J. C., Monk, R., Taylor, A. L., Ozbek, S., Fascetti, N., Baumgartner, S., and Engel, J. (2003) J. Biol. Chem. 282, 479–494
3. Adams, J. C. (2004) Int. J. Biochem. Cell Biol. 36, 1102–1114
4. Carlson, C. B., Bernstein, D. A., Annis, D. S., Misenheimer, T. M., Hannah, B. L., Mosher, D. F., and Keck, J. L. (2005) Nat. Struct. Mol. Biol. 12, 910–914
5. Misenheimer, T. M., Hahr, A. J., Harms, A. C., Annis, D. S., and Mosher, D. F. (2001) J. Biol. Chem. 276, 45882–45887
6. Kvasnakul, M., Adams, J. C., and Hohenester, E. (2004) EMBO J. 23, 1223–1233
7. Misenheimer, T. M., Hannah, B. L., Annis, D. S., and Mosher, D. F. (2003) Biochemistry 42, 5125–5132
8. Arber, S., and Caroni, P. (1995) J. Cell Biol. 131, 1083–1094
9. Lawler, J., Duquette, M., Whittaker, C. A., Adams, J. C., McHenry, K., and DeSimone, D. W. (1993) J. Cell Biol. 120, 1059–1067
10. Stenina, O. I., Desai, S. Y., Krukovets, I., Kight, K., Janigro, D., Topol, E. J., and Plow, E. F. (2003) Circulation 108, 1514–1519
11. Narouz-Ott, L., Maurer, P., Nitschke, D. P., Smyth, N., and Paulsson, M. (2000) J. Biol. Chem. 275, 37110–37117
12. Chen, Y. W., Zhao, P., Borup, R., and Hoffman, E. P. (2000) J. Cell Biol. 151, 1321–1336
13. Adams, J. C., and Lawler, J. (1994) Mol. Cell. Biol. 5, 423–437
14. Topol, E. J., McCarthy, J., Gabriel, S., Molintero, D. J., Rogers, W. J., Newby, L. K., Freedman, M., Metivier, J., Cannata, R., O’Donnell, C. J., Kotik-Marchant, K., Mudrugasen, G., Plow, E. F., Stenina, O., and Daley, G. Q. (2001) Circulation 104, 2641–2644
15. Wessel, J., Topol, E. J., Ji, M., Meyer, J., and McCarthy, J. J. (2004) Am. Heart J. 147, 905–909
16. Cui, J., Randell, E., Renouf, J., Sun, G., Han, F.-Y., Younghusband, B., and Xie, Y.-G. (2004) Arterioscler. Thromb. Vasc. Biol. 24, e183–e184
17. Selander-Sunnerhagen, M., Ullner, M., Persson, E., Teleman, O., Stenflo, J., and Drakenberg, T. (1992) J. Biol. Chem. 267, 10642–10649
18. Mosher, D. F., Hulwiler, K. G., Misenheimer, T. M., and Annis, D. S. (2002) Methods Cell Biol. 69, 69–81
19. Lawler, J., McHenry, K., Duquette, M., and Derick, L. (1995) J. Biol. Chem. 270, 2809–2814
20. Mach, H., Middaugh, C. R., and Lewis, R. V. (1992) Anal. Biochem. 200, 74–80
21. Lawler, J., and Simons, E. R. (1983) J. Biol. Chem. 258, 12098–12101
22. Hannah, B. L., Misenheimer, T. M., Pranghofer, M. M., and Mosher, D. F. (2004) J. Biol. Chem. 279, 51915–51922
23. Chen, H., Deere, M., Hecht, J. T., and Lawler, J. (2000) J. Biol. Chem. 275, 26538–26544
24. Maddox, B. K., Mokashi, A., Keene, D. R., and Bachinger, H. P. (2000) J. Biol. Chem. 275, 11412–11417
25. Thorn, J., Rosenberg, K., Nitsche, D. P., Pihlajamaa, T., Ala-Kokko, L., Heinegard, D., Paulsson, M., and Maurer, P. (2001) J. Biol. Chem. 276, 6683–6692
26. Circoult, T. E. (ed) (1997) Protein Structure, A Practical Approach, 2nd Ed., pp. 287–296, Oxford University Press, Oxford
27. Stenina, O. I., Ustynov, V., Krukovets, I., Marinic, T., Topol, E. J., and Plow, E. F. (2005) JASER J. 19, 1893–1895
28. Whitman, P., Downing, A. K., and Handford, P. A. (1998) Protein Eng. 11, 957–959