Distinct Secondary Structures of the Leucine-rich Repeat Proteoglycans Decorin and Biglycan

GLYCOSYLATION-DEPENDENT CONFORMATIONAL STABILITY

(Received for publication, December 1, 1998, and in revised form, January 13, 1999)

Priya Krishnan‡§, Anne M. Hocking‡§, J. Martin Scholtz‡, C. Nick Pace¶, Kimberly K. Holik‡, and David J. McQuillan‡§

From the ‡Center for Extracellular Matrix Biology, Institute of Biosciences and Technology, Houston Texas 77030-3303 and the ¶Department of Medical Biochemistry & Genetics, Texas A&M University System Health Science Center, College Station, Texas 77843-1114

Biglycan and decorin have been overexpressed in eukaryotic cells and two major glycoforms isolated under native conditions: a proteoglycan substituted with glycosaminoglycan chains; and a core protein form secreted devoid of glycosaminoglycans (Hocking, A. M., Strugnell, R. A., Ramamurthy, P., and McQuillan, D. J. (1996) J. Biol. Chem. 271, 19571–19577; Ramamurthy, P., Hocking, A. M., and McQuillan, D. J. (1996) J. Biol. Chem. 271, 19578–19584). Far-UV CD spectroscopy of decorin and biglycan proteoglycans indicates that, although they are predominantly β-sheet, biglycan has a significantly higher content of α-helical structure. Decorin proteoglycan and core protein are very similar, whereas the biglycan core protein exhibits closer similarity to the decorin glycoforms than to the biglycan proteoglycan form. However, enzymatic removal of the chondroitin sulfate chains from biglycan proteoglycan does not induce a shift to the core protein structure, suggesting that the final form is influenced by polysaccharide addition only during biosynthesis. Fluorescence emission spectroscopy demonstrated that the single tryptophan residue, which is at a conserved position at the C-terminal domain of both biglycan and decorin, is found in similar microenvironments. This indicates that in this specific domain the different glycoforms do exhibit apparent conservation of structure. Exposure of decorin and biglycan to 10 M urea resulted in an increase in fluorescent intensity, which indicates that the emission from tryptophan in the native state is quenched. Comparison of urea-induced protein unfolding curves provide further evidence that decorin and biglycan assume different structures in solution. Decorin proteoglycan and core protein unfold in a manner similar to a classic two-state model, in which there is a steep transition to an unfolded state between 1 and 2 M urea. The biglycan core protein also shows a similar steep transition. However, biglycan proteoglycan shows a broad unfolding transition between 1 and 6 M urea, probably indicating the presence of stable unfolding intermediates.

Decorin and biglycan are small proteoglycans comprising chemically similar core proteins substituted at the N-terminal end with one or two chondroitin/dermatan sulfate chains, respectively. Despite the presumed structural similarity between biglycan and decorin (1), they have distinct patterns of temporal and spatial expression suggesting different functions. They are members of a family of glycoproteins grouped together on the basis of their presence in the extracellular matrix, and by virtue of a leucine-rich motif that dominates the core protein (for review, see Ref. 2). Most of the members of this family exist in tissues as proteoglycans and have been labeled the small leucine-rich proteoglycans (SLRPs) (3).

The protein core of decorin (Fig. 1a) and biglycan (Fig. 1b) can be divided into distinct domains, based on amino acid sequence and specific post-translational modifications: a signal sequence that targets the nascent polypeptide to the secretory route; a short propeptide of highly charged amino acids that undergoes differential tissue- and cell-specific cleavage; an N-terminal glycosaminoglycan attachment region containing one (decorin) or two (biglycan) Ser-Gly dipeptide consensus sequences; a leucine-rich repeat (LRR) domain that represents more than two thirds of the core protein and that is flanked by highly conserved disulfide-bonded cysteine clusters; and a short C-terminal domain. The core protein of decorin has three consensus sites for N-linked oligosaccharides; two of these sites are conserved in biglycan.

The post-translational modifications of decorin and biglycan are complex and variable, wherein differentially glycosylated forms of these molecules have been isolated from tissues and cells. Decorin core protein devoid of a glycosaminoglycan chain has been isolated (4, 5), although it is yet to be demonstrated whether this is due to post-secretory cleavage of the glycosaminoglycan attachment domain, or synthesis and secretion of core protein that bypasses the glycosaminoglycan synthetic machinery. There is evidence that members of the SLRP family may be “part time” proteoglycans. Overexpression of decorin and biglycan yields significant amounts of secreted core protein devoid of glycosaminoglycan chains (6, 7), the proportion of which appears to be cell type-dependent and related to the endogeous activity of xylosyltransferase (8) (the enzyme that catalyzes the first sugar transfer reaction initiating chondroitin sulfate polymerization on a core protein substrate). The decorin core protein is differentially substituted with N-linked oligosacchar-
The LRR is a structural motif first identified by Patthy (10) and subsequently refined by Kobe and Deisenhofer (11), which is usually present in tandem array and has been described in an increasing number of proteins, giving rise to a LRR superfamily. It is likely that the conserved residues of each LRR motif define the secondary structure, while the intervening residues determine specificity of interaction with ligands. In 1993, Kobe and Deisenhofer solved the crystal structure of ribonuclease inhibitor (12), a LRR protein that consists of 15 repeats. This remains the only report of the detailed structure of a LRR protein and may provide a prototype for all LRR proteins. Each LRR consists of a $\beta$-strand parallel to an $\alpha$-helix forming a hairpin structure, which is aligned parallel to a common axis resulting in a non-globular horseshoe-shaped protein. Binding of the ligand to the concave face (i.e. $\beta$-strands) results in a conformational change of the entire structure and increases the available surface area for binding.

The LRR domain of the SLRP family members is unique within the superfamily in that it is flanked by cysteine clusters; at the N-terminal end of the LRR domain there are four similarly spaced cysteine residues in a 20-amino acid stretch that are involved in disulfide bonds; and at the C-terminal end there are two cysteine residues also believed to form an intrachain disulfide bond. The 24-amino acid LRR consensus for members of the SLRP family is $X-X-(I/V/L)-X-X-X-(F/P/L)-X-X-(L/P)-X-X-L-X-(L/I)-X-L-X-X-N-X-(L/I),$ where $X$ is any amino acid, and in the case where more than one amino acid is noted, the first occurs most often (2).

Attempts have been made to predict the structure of decorin (and related molecules) based on the crystal structure of ribonuclease inhibitor. Computer modeling, constrained by parameters established by the structure of ribonuclease inhibitor, have suggested that the decorin core protein forms an arch-shaped protein with the glycosaminoglycan chain and N-linked oligosaccharides situated on the same side of the molecule (1). High magnification rotary shadowing electron micrographs of scleral decorin reveal a similarly "horseshoe-shaped" molecule (13) consistent with the computer modeling prediction. However, the ribonuclease inhibitor is composed entirely of LRRs, whereas the N and C termini of SLRPs have extended non-LRR-containing domains. The inhibitor lacks cysteine clusters flanking the LRR domain, which, through intramolecular disulfide bond formation (14), might provide points of stabilization at either end of the LRR domain. The length of the decorin repeat motif is 24 residues, which is shorter than for ribonuclease inhibitor and may result in a restricted $\beta$-sheet. However, Kajava has recently modeled the LRR superfamily (15), and has predicted that structures with horseshoe curvature are feasible for proteins with shorter leucine-rich repeats, although the different subfamilies may differ significantly in tertiary structure. It is also possible that the extensive glycosylation of SLRPs may influence the folding of the LRR domain.

In the current study, our data indicate that recombinant human decorin and biglycan have different secondary structures in solution and marked differences in conformational stability, as assessed by circular dichroism and fluorescent spectroscopy. Furthermore, we provide evidence that both the conformation and stability of these molecules is variably influenced by whether they are synthesized with or without a glycosaminoglycan chain, whereas removal of polysaccharides after secretion has no appreciable influence on conformation or stability.

**EXPERIMENTAL PROCEDURES**

**Materials—**Ultrapure urea was obtained from Ambion Inc. (Austin, TX). Ultrafree-15 centrifugal filtration devices were from Millipore Corp. (Bedford, MA). All other materials were obtained as described previously (7).

**Protein Purification—**Recombinant decorin and biglycan glycoforms were expressed and purified using the vaccinia virus/T7 bacteriophage expression system, as described previously (6, 7). Briefly, recombinant proteins were purified on a column of iminodiacetic acid immobilized on Sepharose 6B. Proteoglycan and core protein forms were resolved and eluted by a linear gradient of imidazole in column buffer (0.5 M NaCl, 20 mM Tris-HCl, pH 8.0). Pooled fractions were dialyzed against phosphate-buffered saline (PBS), pH 7.4, and concentrated on Ultrafree-15 centrifugal filter devices. Protein concentrations were determined by the molar extinction coefficient (16).

**Stock Solutions—**Urea stock solutions (10 M) in a buffer of 50 mM Na$_2$HPO$_4$/K$_2$HPO$_4$, pH 7.0 (17), were prepared daily for each experiment and filtered (0.22-$\mu$m pore) prior to use. The urea concentration of each stock solution was calculated by weight and by refractive index (17). The buffer solution without urea is referred to as "phosphate buffer."

**Circular Dichroism Spectroscopy—**CD spectra of protein samples were recorded on a Jasco 720 spectropolarimeter using a 2-mm pathlength quartz cell at room temperature at a concentration of 10 $\mu$M in phosphate buffer. The recorded spectra (190–250 nm) were the average of 10 scans and were corrected to background (phosphate buffer alone). Sample cuvettes were sealed with a Teflon stopper so that no evapora-
RESULTS AND DISCUSSION

Recombinant biglycan and decorin were expressed in the vaccinia/T7 bacteriophage system (6, 7). Both biglycan and decorin were synthesized as two glycoforms: a proteoglycan form (Fig. 2) and a core protein form that was secreted devoid of glycosaminoglycan chains. The structure of the core protein forms of biglycan and decorin (18) predicted that the secondary structure of biglycan was 30% α-helix, 44% β-sheet, and 17% random coil, whereas decorin comprised 6% α-helix, 34% β-sheet, and 35% random coil.

After equilibration of recombinant biglycan and decorin in 10 M urea for 16 h, samples were analyzed by CD spectroscopy (Fig. 2, open circles). The CD spectra for both biglycan (Fig. 2a, open circles) and decorin (Fig. 2c, open circles) in 10 M urea reflected a loss of CD signal typical of an absence of secondary structure.

CD spectroscopy was also used to examine the secondary structure of the core protein glycoforms of biglycan and decorin (Fig. 2). The CD spectra for the biglycan core protein in phosphate buffer had a minima at 215 nm (Fig. 2b, solid circles), with a significantly broader curve than seen for the proteoglycan form (Fig. 2a). Computer deconvolution analysis predicted that the secondary structure of biglycan core protein was significantly different to the proteoglycan form (13% α-helix, 36% β-sheet, 19% β-turn, and 37% random coil; Ref. 18). Thus, the biglycan core protein, synthesized devoid of glycosaminoglycan chains, had a secondary structure that is different to the proteoglycan form. The effect of chondroitin sulfate chain addition on the secondary structure of biglycan and decorin core proteins was assessed by digestion with chondroitinase ABC (to remove the glycosaminoglycan chains). There was no detectable difference between the spectra generated from undigested and digested proteoglycans. Glycosaminoglycan chains, at 10-fold higher concentration than in samples analyzed in Fig. 2, did not contribute to the CD spectra (data not shown). Therefore, removal of the glycosaminoglycan chains from secreted proteoglycan does not significantly influence secondary structure.

Comparison of the CD spectra of decorin core protein (Fig. 2d, solid circles) with the spectra of decorin proteoglycan (Fig. 2b, solid circles) showed essentially identical curves with the minima of both glycoforms at 218 nm. The CD scans clearly show that decorin and biglycan have distinct secondary structures. Furthermore, biglycan synthesized and secreted devoid of chondroitin sulfate chains assumes a different structure to biglycan substituted with chondroitin sulfate. However, removal of the bulk of the chondroitin sulfate mass from biglycan after purification had no measurable influence on the structure. Biglycan appears to have more α-helical content in its secondary structure relative to biglycan core and decorin glycoforms, which are primarily β-sheet in structure. Decorin, on the other hand, appears to form the same structure in solution irrespective of substitution with chondroitin sulfate.

Fluorescence Emission Spectroscopy—The mature core protein of biglycan and decorin both have a single tryptophan residue situated between the two conserved cysteine residues at the C-terminal end of the core protein (Fig. 3a). Peptide sequencing of bovine biglycan has shown that these cysteines form an intramolecular disulfide bond (14). Comparison of the amino acid sequence in this region reveals that biglycan and decorin share 65% amino acid identity. Fluorescence spectroscopy was used to analyze the environment of this tryptophan in native and denatured biglycan and decorin. The fluorescent intensity for all four glycoforms increased in the presence of 10 M urea (Fig. 3, b–e, closed circles) relative to the intensity in phosphate buffer (Fig. 3, b–e, solid circles). These data indicate that the emission from the tryptophan in the native glycoforms is quenched. Biglycan proteoglycan in PBS had a maximum emission wavelength of 342 nm (Fig. 2b, open circles), and after...
denaturation the peak emission wavelength was shifted to 352 nm (Fig. 2b, closed circles). The maximum emission wavelength of decorin proteoglycan in PBS was 345 nm (Fig. 2d, solid circles); in 10 M urea, the emission wavelength shifted to 354 nm (Fig. 2d, open circles). These results suggest that the tryptophan in native biglycan and decorin is partially buried; in denatured biglycan and decorin, the tryptophan is exposed to a polar environment.

The magnitude of the peak emission wavelength shift for the native and denatured core protein forms of biglycan (Fig. 3c) was similar to that observed for the proteoglycan form (Fig. 3b). The intrinsic fluorescence spectra for biglycan core protein revealed that the native protein had an emission maxima at 341 nm (Fig. 2c, solid circles), which shifted to 350 nm for the denatured protein (Fig. 2c, open circles). Therefore, it appears that the tryptophan is in a similar environment in both glycoforms of biglycan. The spectra for native decorin core protein had a peak emission wavelength maxima of 350 nm (Fig. 3e, solid circles). The maxima for the fluorescence spectra of decorin core protein in 10 M urea was 355 nm (Fig. 2e, open circles). The peak emission wavelength for decorin is different between core protein (350 nm) and proteoglycan (345 nm), which indicates that the microenvironment of the tryptophan in these two glycoforms may be different. The decorin core protein (Fig. 3e) is more solvent-exposed in the native state relative to the decorin proteoglycan (Fig. 3d). However, taken together, these subtle differences suggest that the C-terminal domain is structured similarly among all four glycoforms.

**Urea Denaturation Curves**—To further characterize structural differences between biglycan and decorin, the conformational stability was investigated. Urea denaturation curves were determined for each glycoform of biglycan and decorin.

The CD spectra (shown in Fig. 2) revealed the maximal CD signal difference between native and denatured proteoglycan was at 220 nm, and this wavelength was used to monitor changes in the CD signal as unfolding occurred. The denaturation curves generated for the proteoglycan forms of biglycan and decorin are complex (Fig. 4), but highly reproducible, and were not amenable to curve fitting algorithms. The denaturation curve for biglycan proteoglycan indicates the protein is very susceptible to urea denaturation based on the limited pretransition baseline from 0–0.5 M urea (Fig. 4a). However, the transition from the folded to unfolded state occurs gradually from 1 to 6 M urea, and it is not clear where the post-transition baseline begins. The unfolding of biglycan proteoglycan probably proceeds through stable intermediates, reflecting sequential disruption of domains. Unfolding of decorin proteoglycan follows a simpler, possibly two-state, mechanism (Fig. 4c), with a pretransition baseline from 0 to 1.0 M urea, a sharp transition between 1.0 and 2.0 M urea, and an apparent post-transition baseline with increasing urea concentration.

The urea-induced unfolding of the core protein glycoforms of biglycan and decorin was also examined. Biglycan core protein (Fig. 4b) had a pretransition between 0 and 1.5 M urea, a sharp transition between 1.5 and 2.5 M, followed by a slowly increasing post-transition above 3.5 M urea. The denaturation curve for decorin core protein was similar to both the biglycan core protein and the decorin proteoglycan glycoforms, with a pretransition region at 0–1.0 M, a transition from 1.0 to 2.0 M urea, and a similar post-transitional baseline at higher urea concentrations (Fig. 4d).

**Reversibility of Unfolding**—Reversibility of unfolding is an important parameter when defining conformational stability. All four glycoforms were equilibrated in 10 M urea and then diluted to a urea concentration of 1.0 M urea, at a final concentration of 10 μM. This preparation was compared with glycoforms (10 μM) that had been equilibrated directly in 1 or 10 M urea. The effect on secondary structure was examined by far-UV CD spectroscopy (Fig. 5). All of the refolding profiles (Fig. 5, triangles) demonstrate that none of the glycoforms were able to refold to their original conformation after exposure to 10 M. The biglycan proteoglycan in PBS (Fig. 2a, solid circles) and 1.0 M urea (Fig. 5a, solid circles) had similar CD profiles, with

**FIG. 3.** Fluorescence emission spectra of recombinant glycoforms. a, amino acid sequence spanning the C-terminal disulfide-bonded cysteine domain of decorin (upper sequence) and biglycan (lower sequence). Conserved residues are indicated by vertical lines, and the conserved tryptophan (W) is boxed. Fluorescence emission spectra are shown for biglycan proteoglycan (b), biglycan core protein (c), decorin proteoglycan (d), and decorin core protein (e), in phosphate buffer (●) and after equilibration in 10 M urea (○). The vertical dashed line is in an identical position in all panels and is provided to assist in curve comparison.

**FIG. 4.** Urea-induced unfolding of biglycan proteoglycan (a), biglycan core protein (b), decorin proteoglycan (e), and decorin core protein (d). Glycoproteins (10 μM) were equilibrated overnight in increasing concentrations of urea, and unfolding monitored by far-UV circular dichroism spectroscopy.
spectra to native proteoglycan (Fig. 5b). Diluted into 1 M urea and allowed to equilibrate overnight in 1 M urea (●) or 10 M urea (○), and 10 M urea for 6 h, then diluted into 1 M urea and allowed to equilibrate for 12 h (▲). A recent study by Font et al. (24) showed that fibromodulin, a member of the SLRP family, exhibited a CD spectra consistent with a predominantly β-sheet structure (minima at ~210 nm). Furthermore, brief exposure to 6 M urea and heat (60 °C) did not irreversibly disrupt the secondary structure. This is in contrast to the present study, in which extended exposure to 10 M urea alone has serious consequences on the conformation of the core protein. Furthermore, thermal denaturation of biglycan or decorin (up to 60 °C) is not reversible (data not shown). This further illustrates that, despite the similarity of SLRP members at the amino acid level, and presumed evolutionary relatedness, there appears to be significant divergence in secondary structure and conformational stability. Functionally, this is likely to be critical to the differential biological activities and distribution of SLRP molecules (2).

Glycosaminoglycans are long extended polymers of repeating disaccharide units. It is not unreasonable to speculate that these large polysaccharides can influence the structural conformation or stability of a protein. In this study, the chondroitin sulfate chains of biglycan may have a critical role in stabilizing the secondary structure of the protein during biosynthesis. Significant differences in the CD spectra of the native biglycan proteoglycan and core protein provide evidence that the presence of glycosaminoglycan chains can alter structure. It is unclear whether the glycosaminoglycan affects the secondary structure and conformational stability. Functionally, this is likely to be critical to the differential biological activities and distribution of SLRP molecules (2).

In summary, comparison of the biophysical properties of biglycan and decorin indicates that these small leucine-rich repeat proteoglycans have different overall secondary structures, as assessed by circular dichroism spectroscopy. However, fluorescence spectroscopy indicates that the conserved tryptophan in the C-terminal disulfide-bonded domain is in a similar environment for both biglycan and decorin. A qualitative analysis of conformational stability revealed the possibility of multiple transitions during urea-induced unfolding of biglycan proteoglycan; in contrast, biglycan core protein and the decorin glycoforms appear to follow a two-state unfolding mechanism. Glycosylation also had differential effects on the structure and stability of biglycan, but not decorin. The core protein form of biglycan is more stable than the proteoglycan, and they appear to assume different structures in solution. This is in contrast to the decorin core protein, which assumes a similar conformation independent of substitution with a glycosaminoglycan chain.

In summary, comparison of the biophysical properties of biglycan and decorin indicates that these small leucine-rich repeat proteoglycans have different overall secondary structures, as assessed by circular dichroism spectroscopy. However, fluorescence spectroscopy indicates that the conserved tryptophan in the C-terminal disulfide-bonded domain is in a similar environment for both biglycan and decorin. A qualitative analysis of conformational stability revealed the possibility of multiple transitions during urea-induced unfolding of biglycan proteoglycan; in contrast, biglycan core protein and the decorin glycoforms appear to follow a two-state unfolding mechanism. Glycosylation also had differential effects on the structure and stability of biglycan, but not decorin. The core protein form of biglycan is more stable than the proteoglycan, and they appear to assume different structures in solution. This is in contrast to the decorin core protein, which assumes a similar conformation independent of substitution with a glycosaminoglycan chain.

A recent study by Font et al. (24) showed that fibromodulin, a member of the SLRP family, exhibited a CD spectra consistent with a predominantly β-sheet structure (minima at ~210 nm). Furthermore, brief exposure to 6 M urea and heat (60 °C) did not irreversibly disrupt the secondary structure. This is in contrast to the present study, in which extended exposure to 10 M urea alone has serious consequences on the conformation of the core protein. Furthermore, thermal denaturation of biglycan or decorin (up to 60 °C) is not reversible (data not shown). This further illustrates that, despite the similarity of SLRP members at the amino acid level, and presumed evolutionary relatedness, there appears to be significant divergence in secondary structure and conformational stability. Functionally, this is likely to be critical to the differential biological activities and distribution of SLRP molecules (2).

Glycosaminoglycans are long extended polymers of repeating disaccharide units. It is not unreasonable to speculate that these large polysaccharides can influence the structural conformation or stability of a protein. In this study, the chondroitin sulfate chains of biglycan may have a critical role in stabilizing the secondary structure of the protein during biosynthesis. Significant differences in the CD spectra of the native biglycan proteoglycan and core protein provide evidence that the presence of glycosaminoglycan chains can alter structure. It is unclear how the glycosaminoglycan affects the secondary structure and conformational stability. Functionally, this is likely to be critical to the differential biological activities and distribution of SLRP molecules (2).
will impact significantly on the biology of these molecules with respect to their role in collagen fibrillogenesis (25, 26), modulation of transforming growth factor-β activity (27–31), and interaction with cell surface receptors (32–34).

Acknowledgments—We thank Dr. Steve Labrenz and Dr. Rebecca Rich for many useful discussions and for assistance with operating the CD and fluorescent spectrophotometers.

REFERENCES

1. Weber, I. T., Harrison, R. W., and Iozzo, R. V. (1996) J. Biol. Chem. 271, 31767–31770
2. Hocking, A. M., Shinomura, T., and McQuillan, D. J. (1998) Matrix Biol. 17, 1–19
3. Iozzo, R. V., and Murdoch, A. D. (1996) FASEB J. 10, 598–614
4. Fleischmajer, R., Fisher, L. W., MacDonald, E. D., Jacobs, L., Jr., Perlish, J. S., and Termine, J. D. (1991) J. Struct. Biol. 106, 82–90
5. Sampaio, L. D. O., Bayliss, M. T., Hardingham, T. E., and Muir, H. (1988) Biochem. J. 254, 757–764
6. Ramamurthy, P., Hocking, A. M., and McQuillan, D. J. (1996) J. Biol. Chem. 271, 19578–19584
7. Hocking, A. M., Strugnell, R. A., Ramamurthy, P., and McQuillan, D. J. (1996) J. Biol. Chem. 271, 19571–19577
8. Glossl, J., Beck, M., and Kresse, H. (1984) J. Biol. Chem. 259, 14144–14150
9. Roughley, P. J., White, R. J., Magny, M. C., Liu, J., Pearce, R. H., and Mort, J. S. (1983) Biochem. J. 205, 421–426
10. Patthy, L. (1987) J. Mol. Biol. 198, 567–577
11. Kobe, B., and Deisenhofer, J. (1994) Trends Biochem. Sci. 19, 415–421
12. Kobe, B., and Deisenhofer, J. (1993) Nature 366, 751–756
13. Scott, J. E. (1996) Biochemistry 35, 8795–8799
14. Neame, P. J., Choi, H. U., and Rosenberg, L. C. (1989) J. Biol. Chem. 264, 8653–8661
15. Kajava, A. V. (1998) J. Mol. Biol. 277, 519–527
16. Pace, C. N., Vajdos, F., Fee, L., Grimsley, G., and Gray, T. (1995) Protein Sci. 4, 2411–2423
17. Pace, C. N., and Laurents, D. V. (1989) Biochemistry 28, 2520–2525
18. Srerama, N., and Woody, R. W. (1993) Anal. Biochem. 209, 32–44
19. Tomsen, P., Alcorn, S. W., and Johnson, W. C., Jr. (1992) Anal. Biochem. 200, 321–331
20. Johnson, W. C., Jr. (1988) Annu. Rev. Biophys. Biophys. Chem. 17, 145–166
21. Perceel, A., Park, K., and Fasman, G. D. (1992) Anal. Biochem. 203, 83–93
22. Provencher, S. W., and Glockner, J. (1981) Biochemistry 20, 33–37
23. Pittsyn, O. B. (1985) Curr. Opin. Struct. Biol. 3, 74–78
24. Font, B., Eichenberger, D., Goldschnidt, D., Boutilier, M. M., and Hulmes, D. J. (1998) Eur. J. Biochem. 254, 580–587
25. Vogel, K. G., Paulson, M., and Heinegard, D. (1984) Biochem. J. 223, 587–597
26. Vogel, K. G., Koob, T. J., and Fisher, L. W. (1987) Biochem. Biophys. Res. Comm. 148, 658–663
27. Border, W. A., Noble, N. A., Yamamoto, T., Harper, J. R., Yamaguchi, Y., Pierschbacher, M. D., and Ruoslahti, E. (1992) Nature 360, 361–364
28. Harper, J. R., Spiro, R. C., Gaarde, W. A., Tamura, R. N., Pierschbacher, M. D., Noble, N. A., Stecker, K. K., and Border, W. A. (1994) Methods Enzymol. 245, 241–254
29. Isaka, Y., Brees, D. K., Ikegaya, K., Kaneda, Y., Imai, E., Noble, N. A., and Border, W. A. (1996) Nat. Med. 2, 418–423
30. Ruoslahti, E., Yamaguchi, Y., Hildebrand, A., and Border, W. A. (1992) Cold Spring Harbor Symp. Quant. Biol. 57, 309–315
31. Border, A. K., Flanagan, M., Matsu, T., Hildebrand, D., C. R., and Ruoslahti, E. (1994) Biochem. J. 302, 527–534
32. De Luca, A., Santina, M., Baldi, A., Giordano, A., and Iozzo, R. V. (1996) J. Biol. Chem. 271, 18961–18965
33. Moscatello, D. K., Santina, M., Mann, D. M., McQuillan, D. J., Wong, A. J., and Iozzo, R. V. (1998) J. Clin. Invest. 101, 406–412
34. Patel, S., Santina, M., McQuillan, D. J., Iozzo, R. V., and Thomas, A. P. (1998) J. Biol. Chem. 273, 3121–3124