Christner, J. A., Münck, E., Janick, P. A., & Siegel, L. M. (1981) *J. Biol. Chem. 256*, 2098-2101.
Christner, J. A., Münck, E., Janick, P. A., & Siegel, L. M. (1983a) *J. Biol. Chem. 258*, 11147-11156.
Christner, J. A., Janick, P. A., Siegel, L. M., & Münck, E. (1983b) *J. Biol. Chem. 258*, 11157-11164.
Christner, J. A., Münck, E., Kent, T. A., Janick, P. A., Salerno, J. C., & Siegel, L. M. (1984) *J. Am. Chem. Soc. 106*, 6786-6794.
Cline, J. F., Janick, P. A., Siegel, L. M., & Hoffman, B. M. (1985) *Biochemistry 24*, 7942-7947.
Day, E. P., Kent, T. A., Lindahl, P. A., Münck, E., Orme-Johnson, W. H., Roder, H., & Roy, A. (1987) *Biophys. J. 52*, 837-853.
De Vries, S., & Albracht, S. P. J. (1979) *Biochim. Biophys. Acta 546*, 334-340.
Janick, P. A., & Siegel, L. M. (1983) *Biochemistry 22*, 504-515.
Janick, P. A., Rueger, D. C., Krueger, R. J., Barber, M. J., & Siegel, L. M. (1983) *Biochemistry 22*, 396-408.
Lancaster, J. R., Vega, J. M., Kamin, H., Orme-Johnson, N. R., Orme-Johnson, W. H., Krueger, R. J., & Siegel, L. M. (1979) *J. Biol. Chem. 254*, 1268-1272.
Lancaster, J. R., Batie, C. J., Kamin, H., & Knaff, D. B. (1982) in *Methods in Chloroplast Molecular Biology* (Edelman, J., Halleck, K. B., & Chua, N.-H., Eds.) pp 723-734, Elsevier Biomedical, Amsterdam.
McRee, D. E., Richardson, D. C., Richardson, J. S., & Siegel, L. M. (1986) *J. Biol. Chem. 261*, 10277-10281.
Münck, E. (1982) in *Iron-Sulfur Proteins* (Spiro, T. G., Ed.) pp 147-175, Wiley, New York.
Murphy, M. J., Siegel, L. M., Tove, S. R., & Kamin, H. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 612-616.
Petersson, L., Cammack, R., & Rao, K. K. (1980) *Biochim. Biophys. Acta 622*, 18-24.
Salerno, J. C., & Leigh, J. S. (1984) *J. Am. Chem. Soc. 106*, 2156-2159.
Siegel, L. M. (1978) in *Mechanisms of Oxidizing Enzymes* (Singer, T. P., & Ondarza, R. N., Eds.) pp 201-214, Elsevier, New York.
Siegel, L. M., & Davis, P. S. (1974) *J. Biol. Chem. 249*, 1587-1598.
Siegel, L. M., Murphy, M. J., & Kamin, H. (1973) *J. Biol. Chem. 248*, 251-264.
Siegel, L. M., Rueger, D. C., Barber, M. J., Krueger, R. J., Orme-Johnson, N. R., & Orme-Johnson, W. H. (1982) *J. Biol. Chem. 257*, 6343-6350.
Vega, J. M., & Kamin, H. (1977) *J. Biol. Chem. 252*, 896-909.
Wilkerson, J. O., Janick, P. A., & Siegel, L. M. (1983) *Biochemistry 22*, 5048-5054.
Young, L. J., & Siegel, L. M. (1988) *Biochemistry* (in press).

Effect of Unfolding on the Tryptophanyl Fluorescence Lifetime Distribution in Apomyoglobin

E. Bismuto,[‡] E. Gratton,[§] and G. Irace*[,‡]

Cattedra di Chimica e Propedeutica Biochimica, Istituto di Chimica e Chimica Biologica, I Facoltà di Medicina e Chirurgia, Università di Napoli, Via Costantinopoli 16, 80138 Napoli, Italy, and Department of Physics, University of Illinois at Urbana-Champaign, 1110 West Green Street, Urbana, Illinois 61801

Received August 5, 1987; Revised Manuscript Received October 16, 1987

ABSTRACT: Proteins exhibit, even in their native state, a large number of conformations differing in small details (substates). The fluorescence lifetime of tryptophanyl residues can reflect the microenvironmental characteristics of these subconformations. We have analyzed the lifetime distribution of the unique indole residue of tuna apomyoglobin (Trp A-12) during the unfolding induced by temperature or guanidine hydrochloride. The results show that the increase of the temperature from 10 to 30 °C causes a sharpening of the lifetime distribution. This is mainly due to the higher rate of interconversion among the conformational substates in the native state. A further temperature increase produces partially or fully unfolded states, resulting in a broadening of the tryptophanyl lifetime distribution. The data relative to the guanidine-induced unfolding show a sigmoidal increase of the distribution width, which is due to the transition of the protein structure from the native to the random-coiled state. The broadening of the lifetime distribution indicates that, even in the fully unfolded protein, the lifetime of the tryptophanyl residues is influenced by the protein matrix, which generates very heterogeneous microenvironments.

Guanidine hydrochloride is a powerful denaturing agent for proteins. Native globular proteins usually undergo a marked structural transition in the presence of guanidine. Generally, the transition is completed at a denaturant concentration ranging between 6 and 8 M at room temperature, except for some exceptionally stable proteins (Pace, 1975). When the transition is completed, proteins are found to be random coiled, without any residual ordered structure (Tanford, 1968). In these conditions, the spectroscopic properties of the aromatic residues are supposed to be similar to those of the monomeric amino acids in water (Demchenko, 1986).

A protein molecule, in a particular conformational state, can assume a very large number of substates, rapidly interconverting at room temperature, having the same coarse overall structure but differing in small structural details. For example,

[‡] Università di Napoli.
[§] University of Illinois at Urbana-Champaign.

0006-2960/88/0427-2132$01.50/0

a side chain may have rotated, some hydrogen bonds may have shifted, a single helix may be displaced, or small movements may occur involving entire structural domains (Austin et al., 1975; Frauenfelder et al., 1979; Hartmann et al., 1982; Ringe et al., 1985). Recently, a hierarchical model for such substates has been proposed, each level of the hierarchy being characterized by a specific interconversion time scale and energy (Ansari et al., 1985, 1987; Frauenfelder & Gratton, 1985; Kuriyan et al., 1987).

The emission decay properties of the tryptophanyl residue depend on the number of protein conformations, on the mobility around each conformation, and on the interconversion rate among conformations (Engh et al., 1986; James et al., 1985; Petrich et al., 1983; Creed, 1984; Szabo et al., 1980). The usual analysis of the fluorescence decay consists of determining the number and the relative amplitude of exponential components. Each component is then associated with a different protein conformation. Recently, it has been shown that the decay is better represented by a continuous distribution of decay rates rather than by the sum of exponentials (Gratton et al., 1986). Static as well as dynamic considerations justify the use of continuous distributions in the analysis of the fluorescence decay rates of proteins (Alcala et al., 1987a,b). The interpretation of the distribution lifetime analysis is based on the assumption that the width of distribution can be correlated with the degree of heterogeneity of tryptophanyl environments.

In this paper, we have analyzed the tryptophanyl lifetime distribution of a protein containing a single indole residue, i.e., tuna apomyoglobin (Colonna et al., 1983), under different conditions perturbing the unfolding equilibrium. The lifetime determinations were performed by multifrequency phase and modulation fluorometry using the harmonic content of a mode-locked laser, which is known to be a valid alternative to the use of time-correlated photon counting techniques (Alcala et al., 1985). The results indicate that the protein unfolding is associated with a progressive broadening of the tryptophanyl lifetime distribution which can be related to a larger number of conformational substates in the unfolded state.

Materials and Methods

Tuna myoglobin was prepared from the heart ventricle of adult bluefin tuna (*Thunnus thynnus*) according to the method previously described (Balestrieri et al., 1978) and further purified by gel filtration on a Sephadex G-50 Superfine column (2.5 cm × 100 cm), followed by ion-exchange chromatography on a Sepharose S column (2.0 cm × 40 cm) to isolate the main component. The Sepharose S column was developed by a linear salt gradient of 0–0.5 M NaCl added to 5 mM phosphate, pH 6.2 (500 mL for each vessel). The apoprotein was prepared by the butanone extraction technique (Teale, 1959) and purified in order to remove aggregated protein on a Sephadex G-25 Superfine column (1.5 cm × 50 cm). The final buffer was always 0.05 M sodium phosphate in 0.1 M NaCl at pH 7. The molar extinction coefficient at 280 nm of apomyoglobin was calculated from the tryptophan and tyrosine content by using extinction coefficients of 5500 and 1250 M^{-1} cm^{-1}, respectively (Wetlaufer, 1962), and was found to be 8000 M^{-1} cm^{-1}. Ultrapure guanidine hydrochloride was obtained from Schwarz/Mann.

Steady-state fluorescence spectra were recorded by using a Perkin-Elmer MPF-44A spectrofluorometer equipped with a thermostated cell holder. Lifetime measurements were performed by a multifrequency cross-correlation phase and modulation fluorometer which uses the harmonic content of a high repetition rate mode-locked Nd-YAG laser. This laser is used to synchronously pump a dye laser whose pulse train is frequency doubled with an angle-tuned frequency doubler (Alcala et al., 1985). The ultraviolet light was continuously tunable over the range 280–310 nm. We choose excitation at 295 nm to eliminate the contribution of tyrosyl residues to the total fluorescence. The emission was observed through a long-wave pass filter (WG 330) with a cutoff wavelength at 330 nm to avoid Raman emission. The modulation frequency was variable from 4 to 250 MHz. A solution of *p*-terphenyl (from Kodak) in cycloexane was placed in the reference cell to correct for "color error" (Lakowicz et al., 1981). A lifetime of 1.000 ns was assigned to the reference solution. An excitation polarizer at 55° with respect to the horizontal plane was used to eliminate polarization effects. Usually, 10 different modulation frequencies were used, and the data were collected until the standard deviations from each measurement of phase and modulation were below 0.2 and 0.004, respectively. The temperature of the sample compartment was controlled by using an external bath circulator (Neslab Model LT 50). The sample temperature was measured prior to and after each measurement in the sample cuvette by using a digital thermometer (Omega, Model 410 B-TC). The observed phase shifts and modulation values were analyzed in terms of Lorentzian lifetime distributions by using the algorithm described elsewhere (Alcala et al., 1987b).

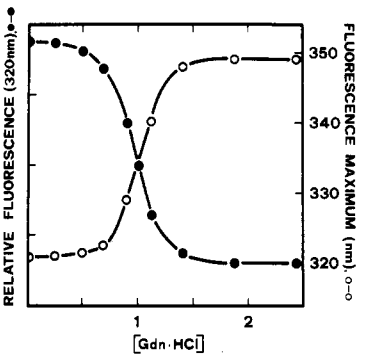

FIGURE 1: Dependence of the tryptophanyl emission at 320 nm and the emission maximum of the bluefin tuna apomyoglobin on guanidine hydrochloride concentration. All solutions contained 5×10^{-2} M phosphate (pH 7.0) and 0.1 M KCl. Protein absorbance at the excitation wavelength (295 nm) was lower than 0.1. The fluorescence was followed with time until an apparent equilibrium was reached.

Results

The emission spectrum at 20 °C of tuna apomyoglobin excited at 298 nm shows, in the absence of denaturant, an emission maximum centered at 321 nm. The exposure of the bluefin tuna apomyoglobin samples to increasing guanidine hydrochloride concentration produces changes both in the fluorescence magnitude and in the position of the emission maximum. Figure 1 shows the dependence on guanidine concentration of the steady-state fluorescence intensity at 320 nm and the position of the emission maximum of tuna apomyoglobin. A sigmoidal curve is observed for both the intensity and the maximum wavelength. At a guanidine concentration as high as 1.5 M, the maximum is shifted to about 350 nm, which corresponds to that observed for *N*-acetyltryptophanamide (NATA)[1] in aqueous solution. At this level of guanidine, tuna apomyoglobin is fully unfolded as documented by far- and near-ultraviolet circular dichroism and fluorescence

[1] Abbreviations: NATA, *N*-acetyltryptophanamide; Gdn·HCl, guanidine hydrochloride; FWHM, full width at half-maximum.

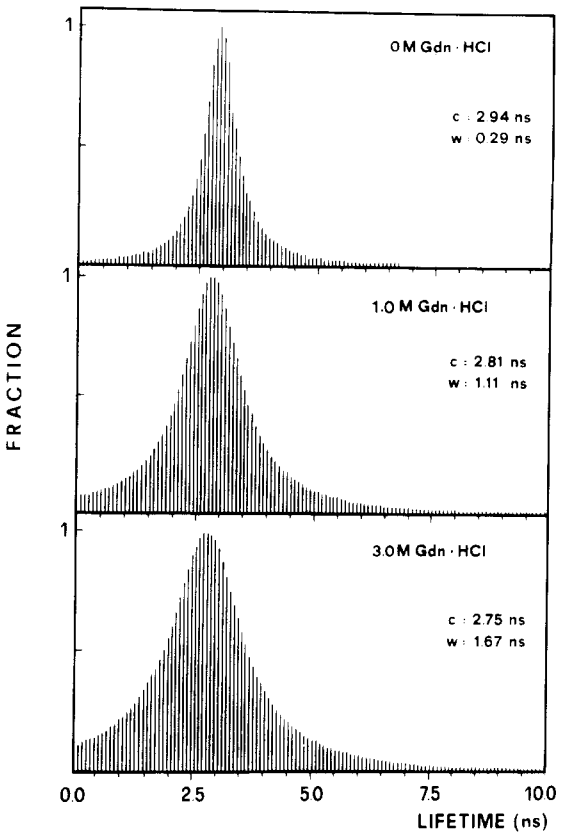

FIGURE 2: Lifetime analysis using a single Lorentzian distribution for tuna apomyoglobin in the presence of 0, 1.0, and 3.0 M guanidine hydrochloride at 10 °C. All solutions contained 0.01 M phosphate–0.1 M NaCl, pH 7.0. Excitation was at 295 nm; emission was observed through a WG 330 filter.

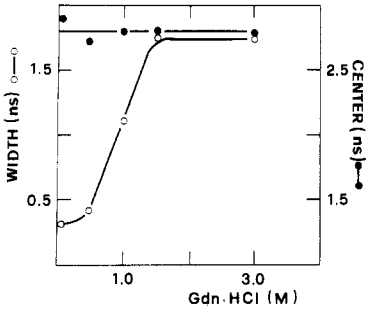

FIGURE 3: Effect of increasing concentration of guanidine on the width and center of the continuous lifetime distribution of tuna apomyoglobin. The experimental details are those reported in Figure 2.

polarization studies (Balestrieri et al., 1978; Colonna et al., 1982; Irace et al., 1986).

The phase and modulation data, collected at several frequencies ranging between 4 and 250 MHz for each guanidine concentration, have been analyzed in terms of continuous unimodal Lorentzian distributions. We have selected this type of distribution since a recently reported comparison among uniform, Gaussian, and Lorentzian distributions, for the analysis of the tryptophanyl emission data in several proteins, showed that Lorentzian distributions better describe the observed emission decay (Gratton et al., 1986). The Lorentzian distribution is characterized by two parameters, i.e., the center and the full width at half-maximum (FWHM) (Bevington, 1969). The distributions were normalized and defined only in the positive lifetime domain. Figure 2 shows the distributions obtained in the presence of 0, 1.0, and 3.0 M guanidine. The increase of guanidine concentration produced a progressive widening of the lifetime distribution accompanied by small

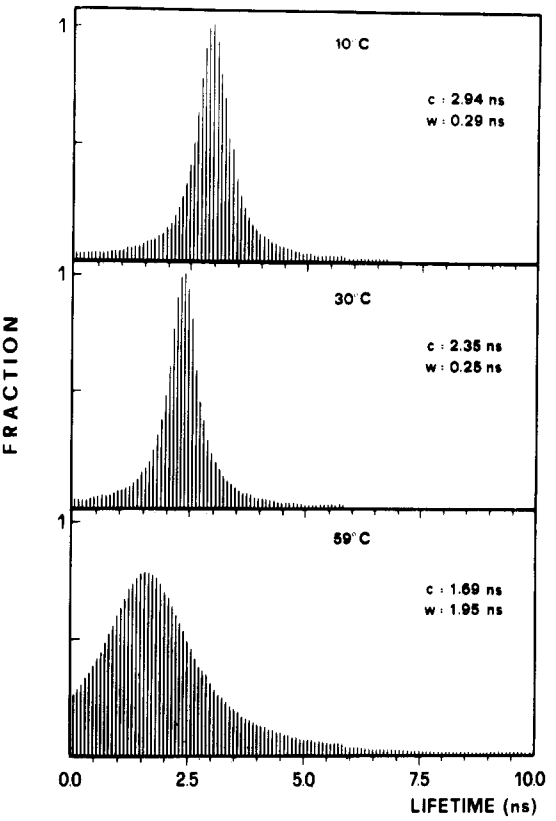

FIGURE 4: Lifetime analysis using a single Lorentzian distribution for tuna apomyoglobin at the indicated temperatures. The other experimental conditions are reported in Figure 2.

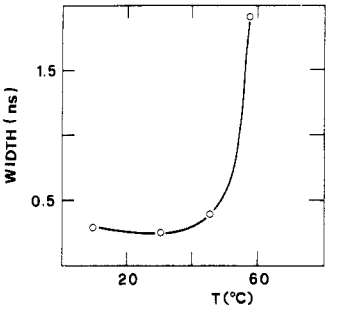

FIGURE 5: Temperature dependence of the width of the continuous lifetime distribution of tuna apomyoglobin. The experimental conditions are those reported in Figure 4.

changes of the distribution center (Figure 3). The dependence of the FWHM on guanidine concentration (Figure 3) is qualitatively similar to the behavior obseryed by following the steady-state fluorescence parameters (Figure 1). A possible explanation for the augmented fluorescence heterogeneity is that the protein can assume unfolded or partially unfolded structures having a larger number of conformational substates.

The temperature dependence of the tryptophan lifetime distribution of tuna apomyoglobin was also investigated. The lifetime distributions at three different temperatures are shown in Figure 4. The dependence of the FWHM on temperature is shown in Figure 5. Two opposed effects on the width of the lifetime distribution are observed. Between 10 and 30 °C, the FWHM decreased from 0.288 to 0.243 ns, whereas at higher temperatures a broadening of the lifetime distribution occurred.

The effect of temperature on the tryptophanyl lifetime distribution in the fully unfolded protein structure was studied by performing the lifetime measurements in the presence of 3 M guanidine hydrochloride. Figure 6 shows the results

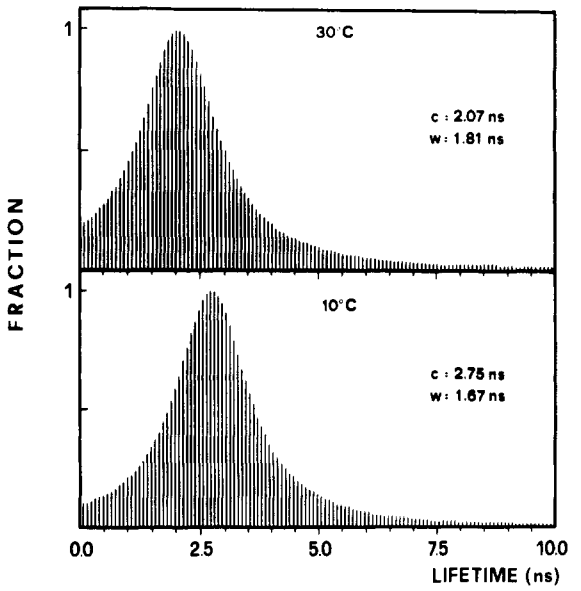

FIGURE 6: Lifetime analysis using a single lifetime distribution for tuna apomyoglobin at 10 and 30 °C in the presence of 3.0 M guanidine hydrochloride. The other experimental details are reported in Figure 2.

obtained at 10 and 30 °C. No substantial change in the FWHM was observed in the presence of the denaturant.

Discussion

The unfolding of apomyoglobin is a multistate process involving the occurrence of at least a molecular intermediate unable to bind the heme (Colonna et al., 1982; Bismuto et al., 1983; Irace et al., 1986; Ragone et al., 1987). The equilibria among the native, intermediate, and denatured states may be perturbed by several denaturing agents, e.g., temperature, pH, urea, and guanidine. Guanidine hydrochloride unfolds the protein by diminishing the magnitude of the hydrophobic effect probably as an indirect result of its effect on the thermodynamic and dynamic properties of water surrounding the protein matrix [for a comprehensive review, see Ghelis (1982)]. The observation that guanidine produces a progressive broadening of the lifetime distribution indicates that the tryptophanyl residue, in the unfolded as well as in the intermediate states, experiences a wider variety of environments during the excited state. This effect suggests the existence of a large conformational space region accessible to the segment of the protein containing the tryptophan. In fact, Alcala et al. (1987b) have recently explained the origin of the tryptophan lifetime distribution in proteins assuming that the indole residue occupies a potential energy well with a quasi-continuum of substates. This potential well represents a stable conformation about which the conformational coordinates of the indole residue fluctuate. The width of the potential well determines the degree of freedom of the residue in the conformational space. In this model, a lifetime distribution can be obtained by associating each substate with a different lifetime value. This seems quite reasonable since each protein subconformation provides a local tryptophanyl environment which strongly influences the emission decay rates (Alcala et al., 1987b,c). Therefore, the observed broadening of the lifetime distribution suggests that the potential energy well becomes wider upon partial or full unfolding.

Temperature influences differently the two most important factors responsible for the protein stability, i.e., hydrogen bonds and hydrophobic interactions. A temperature increase produces weakening of hydrogen bonds but strengthens the hydrophobic interactions (Ghelis & Yon, 1982). The latter interactions increase up to a certain temperature, above which they decrease. Thus, a folded protein is stable only in a certain temperature range. The dependence of the FWHM on the temperature suggests that, in the native form, a temperature increase causes a faster interconversion among the substates which leads to a sharpening of the lifetime distribution. At higher temperature, thermal unfolding produces a broadening of the lifetime distribution. The broadening of the lifetime distribution can be explained by assuming that the tryptophanyl residue has, in the thermally unfolded states, a larger variety of environments with respect to the native conformation. This finding also agrees with heat capacity studies that indicated a larger heat capacity in the denatured form with respect to the native form (Cooper, 1984). The lifetime distributions, observed for both the native and denatured form, confirm the picture that these states are composed of a broad populations of substates.

Finally, the assumption that the spectroscopic properties of a denatured protein are similar to those of the free chromophores in water might not be realistic. In fact, the lifetime distribution of the tryptophan as well as NATA is very narrow, i.e., a FWHM lower than 0.1 ns (Gratton et al., 1986), compared to that observed here for the fully unfolded protein.

References

Alcala, R., Gratton, E., & Jameson, D. M. (1985) *Anal. Instrum. (N.Y.) 14*, 225–250.

Alcala, R., Gratton, E., & Prendergast, F. (1987a) *Biophys. J. 51*, 587–596.

Alcala, R., Gratton, E., & Prendergast, F. (1987b) *Biophys. J. 51*, 597–604.

Alcala, R., Gratton, E., & Prendergast, F. (1987c) *Biophys. J. 51*, 925–936.

Ansari, A., Berendzen, J., Bowne, S. F., Frauenfelder, H., Iben, I. E., Sauke, T. B., Shyamsunder, E., & Young, R. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 5000–5004.

Ansari, A., Berendzen, J., Braunstein, D., Cowen, B. R., Frauenfelder, H., Hong, K. M., Iben, I., Johnson, B. J., Ormos, P., Sauke, T. B., Scholl, R., Schulte, A., Steinbach, P. J., Vittitow, J., & Young, R. D. (1987) *Biophys. Chem. 26*, 337–355.

Austin, R. H., Beeson, K. W., Eisenstein, L., Frauenfelder, H., & Gunsalus, I. C. (1975) *Biochemistry 14*, 5355–5365.

Balestrieri, C., Colonna, G., Giovane, A., Irace, G., Servillo, L., & Tota, B. (1978) *Comp. Biochem. Physiol., B: Comp. Biochem. 60B*, 195–199.

Bevington, P. R. (1969) *Data Reduction and Error Analysis in Physical Science*, p 336, McGraw-Hill, New York.

Bismuto, E., Colonna, G., & Irace, G. (1983) *Biochemistry 22*, 4165–4170.

Colonna, G., Balestrieri, C., Bismuto, E., Servillo, L., & Irace, G. (1982) *Biochemistry 21*, 212–215.

Colonna, G., Irace, G., Bismuto, E., Servillo, L., & Balestrieri, C. (1983) *Comp. Biochem. Physiol., A 76A*, 481–485.

Cooper, A. (1984) *Prog. Biophys. Mol. Biol. 44*, 181–214.

Creed, D. (1984) *Photochem. Photobiol. 39*, 537–562.

Demchenko, A. P. (1986) *Ultraviolet Spectroscopy of Proteins*, p 312, Springer-Verlag, Berlin.

Eftkins, M. (1983) *Biophys. J. 43*, 323–334.

Engh, R. A., Chen, L., & Fleming, G. R. (1986) *Chem. Phys. Lett. 126*, 365–372.

Frauenfelder, H., & Gratton, E. (1985) *Methods Enzymol. 127*, 471–492.

Frauenfelder, H., Petsko, G. A., & Tsernoglou, D. (1979) *Nature (London) 280*, 558–563.

Ghelis, C., & Yon, J. (1982) *Protein Folding*, p 562, Academic, New York.
Gratton, E., Alcala, R., Marriott, G., & Prendergast, F. (1986) in *Progress and Challenges in Natural and Synthetic Polymer Research* (Kwabata, C., & Bishop, A., Eds.) Ohmshu, Tokyo.
Hartmann, H., Parak, F., Steigmann, W., Petsko, G. A., Ponzi, R. D., & Frauenfelder, H. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4967–4971.
Irace, G., Bismuto, E., Savy, F., & Colonna, G. (1986) *Arch. Biochem. Biosphys. 244*, 459–464.
James, D., & Ware, W. (1985) *Chem. Phys. Lett. 120*, 455–459.
Kuriyan, J., Wilz, S., Karplus, M., & Petsko, G. A. (1986) *J. Mol. Biol. 192*, 133.
Lakowicz, J. R., Cherek, H., & Balter, A. (1981) *J. Biochem. Biophys. Methods 15*, 131–146.
Pace, C. N. (1975) *CRC Crit. Rev. Biochem. 3*, 1–43.
Ragone, R., Colonna, G., Bismuto, E., & Irace, G. (1987) *Biochemistry 26*, 2130–2134.
Szabo, G. A., & Rayner, D. M. (1980) *J. Am. Chem. Soc. 102*, 554–563.
Tanford, C. (1968) *Adv. Protein Chem. 23*, 121–282.
Teale, F. W. J. (1959) *Biochim. Biophys. Acta 35*, 543.
Wetlaufer, D. B. (1962) *Adv. Protein Chem. 17*, 303–390.

Structural Characterization of Heparan Sulfate Proteoglycan Subclasses Isolated from Bovine Aortic Endothelial Cell Cultures†

Michael G. Kinsella* and Thomas N. Wight
Department of Pathology SM-30, School of Medicine, University of Washington, Seattle, Washington 98195
Received May 28, 1987; Revised Manuscript Received September 25, 1987

ABSTRACT: Labeled heparan sulfate proteoglycans (HSPG) were isolated from wounded and confluent cultures of bovine aortic endothelial cells by nondegradative extraction with 4 M guanidine hydrochloride and detergent. HSPG were separated from more highly charged chondroitin or dermatan sulfate proteoglycans by ion-exchange chromatography, and subclasses of different hydrodynamic size were isolated by gel filtration. Three major subclasses of HSPG were characterized structurally with respect to the presence and relative size of protein core, the presence and amount of nonsulfated oligosaccharide, and size and structure of heparan sulfate (HS) chains. The largest (600–800-kDa) HSPG subclass (I), isolated from cell layers and media of confluent cultures, bears 38-kDa HS chains on an apparently heterogeneous class of relatively large glycoprotein cores. HSPG II (150–200 kDa), isolated from cell layer or media, has 22-kDa HS chains and smaller core glycoproteins (<50 kDa). HSPG III, the subclass of smallest hydrodynamic size, has 13-kDa HS chains and a glycopeptide core of less than 15 kDa. All subclasses bear varying proportions of non-sulfated oligosaccharides of similar sizes. Comparisons of HS chain structure indicated that the different subclasses have similar proportions (49–55%) of *N*-sulfate, with both *O*-sulfate and highly N-sulfated blocks of disaccharide distributed similarly along HS chains. In addition, HS chains from subclasses II and III contain sequences that are insensitive to periodate oxidation or heparitinase digestion, suggesting that they contain increased proportions of iduronate. HSPG isolated from wounded cultures were structurally similar to those isolated from confluent cultures, except the lowest M_r subclass (HSPG III) has longer HS chains (22 kDa) and little or no associated protein. Like the 22-kDa HS II chains isolated from confluent cultures, media HS III chains isolated from wounded cultures contained a heparitinase-insensitive portion, similar in size to the heparitinase-resistant portion of the 13-kDa HS III chain from confluent cultures.

Vascular endothelial cells synthesize a variety of connective tissue macromolecules including both heparan sulfate proteoglycans (HSPG)[1] and chondroitin or dermatan sulfate proteoglycans (Kramer et al., 1982; Oohira et al., 1983). Although the functions of proteoglycans in endothelial cell biology are incompletely understood, several studies suggest a role for HSPGs in the binding of anticoagulant factors (Marcum et al., 1986) and lipoprotein lipase (Shimda et al., 1981; Cheng et al., 1981) to the endothelial cell surface and as the putative source of "heparin-like" molecules that may be involved in growth control (Castellot et al., 1981). In addition, HSPGs are incorporated into the structure of basement membranes, where they may influence vascular permeability (Kanwar & Farquhar, 1979). More generally, HSPG is associated with the cell surface of many types of cells, either integrated into the plasma membrane or bound to a receptor (Kjellén et al., 1980; Norling et al., 1981; Rapraeger & Bernfield, 1983). Cell surface HSPG associated with other matrical molecules such as fibronectin (Hayman et al., 1982;

† This work was supported by a grant-in-aid from the American Heart Association and NIH Grant HL 18645 (T.N.W.).
* Author to whom correspondence should be addressed.

[1] Abbreviations: BAEC, bovine aortic endothelial cells; HSPG, heparan sulfate proteoglycan; HS, heparan sulfate; Tris, tris(hydroxymethyl)aminomethane; Gdn-HCl, guanidine hydrochloride; glcN, glucosamine; glcUA, glucuronic acid; idoUA, iduronic acid; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; GAG, glycosaminoglycans; CS, chondroitin sulfate; DSPG, dermatan sulfate proteoglycan; PMSF, phenylmethanesulfonyl fluoride.

0006-2960/88/0427-2136$01.50/0