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Two New Bifunctional Protein Modification Reagents and Their Application to the Study of Parvalbumin†

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ABSTRACT: 2-[(Trifluoroacetoxy)mercuri]-4-fluorophenol (MFP) and 4-(acetoxymercuri)phenyl azide (MPA) have been prepared and characterized. The sulfhydryl-specific reagents MFP and MPA have been used to study the structure of parvalbumin. The ^{19}F resonance of the derivative of parvalbumin with MFP was studied at 94.6 and 235.2 MHz. At 94.6 MHz, the ^{19}F signal line width is 25 Hz and T_1 = 0.28 s, while at 235.2 MHz, the line width is 35 Hz and T_1 = 0.39 s. T_1 was not affected by substitution of D_2O for H_2O as a solvent. Removal of calcium from the parvalbumin derivative resulted in the upfield shift of the ^{19}F signal and a decrease in T_1 at 94.6 MHz. The pK_a for phenolic titration of the MFP derivative was 10.75 compared with 9.5 for the free reagent. These results are interpreted in terms of lodgment of the aryl group in a cleft in the surface of the parvalbumin. The derivative with MPA was photolyzed and subjected to amino acid analysis. Comparison of the analysis of parvalbumin, parvalbumin–MPA, and the photolyzed product indicated destruction of aspartic acid during the photolysis. Asp-22 is a reasonable candidate for the residue attacked, based on comparison with the published crystal structure of parvalbumin.

Sulfhydryl groups show an affinity for free or nonsubstituted mercuric ions, whence the name mercaptan (mercuri aptum, fitted for mercury) is derived. Dissociation constants for CH_3Hg^+ with RS^- are in the range of 10^9 times lower than those for CH_3Hg^+ with other ligands, making alkyl or aryl mercury reagents, in general, good candidates for selective labeling of sulfhydryl groups in proteins. In this paper, we present the synthesis of two reagents, 2-[(trifluoroacetoxy)-mercuri]-4-fluorophenol (MFP)[1] and 4-(acetoxymercuri)-phenyl azide (MPA), and explore their usefulness in the study of the structure of parvalbumin, a calcium-binding protein from vertebrate muscle. The fluorinated mercuriphenol was designed with the idea of introducing a fluorinated phenol into

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[1] Abbreviations: Me_2SO-d_6, dimethyl-d_6 sulfoxide; IR, infrared; MFP, 2-[(trifluoroacetoxy)mercuri]-4-fluorophenol; MPA, 4-(acetoxymercuri)phenyl azide; NMR, nuclear magnetic resonance; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; TFA, trifluoroacetic acid; GSH, glutathione.

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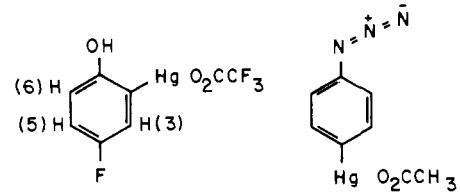

2-trifluoroacetoxymercuri-4-flourophenol (MFP)

4-acetoxymercuri-phenylazide

the protein and then observing the ^{19}F resonance. Possible experiments include the observation of the chemical shift, T_1 and T_2 relaxation times, Overhauser effects, and the pK_a of the phenolic group as a function of the state of the protein. In the case of MPA, it was envisioned that the azide group could be photolyzed after attachment of the azidophenylmercuri group to the sulfhydryl and that the chemical consequences of the insertion of the resultant nitrene could be investigated.

Experimental Procedures

Mercuric Trifluoroacetate. Red mercuric oxide (5.0 g, 0.02 mol) (Aldrich Chemical Co.) was powdered in a mortar and dissolved in a slight molar excess (12 mL) of trifluoroacetic acid (VWR Scientific Co.). The solution was allowed to stand uncovered and then filtered to collect colorless opaque crystals of mercuric trifluoroacetate, yield near-quantitative.

2-[(Trifluoroacetoxy)mercuri]-4-fluorophenol. Mercuration was performed by the method of Dunker et al. (1939). *p*-Fluorophenol (1.4 g, 0.0116 M) (Aldrich Chemical Co.) was dissolved in 20 mL of water. To this solution was added, with stirring, a solution of 5 g (0.0113 M) of mercuric trifluoroacetate in 10 mL of water containing a few drops of trifluoroacetic acid. The solution was left at room temperature for 4 days. Crystals of the product began appearing the first day. The product was collected by filtration and washed with a small amount of water acidulated with trifluoroacetic acid: yield 2.1 g (51.8%); melting point 195–203 °C dec. Anal. Calcd: C, 22.6; H, 0.9; F, 17.9. Found: C, 21.9; H, 0.9; F, 17.9.

The ^1H and ^{19}F NMR spectra were consistent with the proposed structure and showed no impurities. MFP in solution in Me_2SO shows a UV absorption maximum at 280 nm, with $\epsilon = 2800\ M^{-1}\ cm^{-1}$. The extinction coefficient falls to 325 M^{-1} cm^{-1} at 254 nm and 254 M^{-1} cm^{-1} at 248 nm (minimum).

4-(Acetoxymercuri)phenyl Azide. Powdered *p*-(acetoxymercuri)aniline (5.2 g, 0.15 mol) was dissolved in 20 mL of concentrated trifluoroacetic acid by heating at 40–50 °C and stirring vigorously for several hours. The resulting solution was diluted by gradual addition of distilled water, while continuing to stir vigorously, to a total volume of 100 mL. The solution was chilled to 5 °C in an ice–salt bath at which time some of the amine salt began to precipitate, and diazotization was carried out by dropwise addition of 1.38 g (0.02 mol) of sodium nitrite in 25 mL of distilled water. The pH of the solution was about 2.0. If it is lower, it should be raised to 2.0 at this point, because below pH 1.5 the azide product is unstable. Subsequent reactions were carried out in subdued light, and the reaction vessels were wrapped with aluminum foil to exclude light. To the diazotized amine was added dropwise a chilled solution of 1.8 g (0.027 mol) of sodium azide (Pfaltz and Bauer Co.). Nitrogen was immediately evolved, and a dark brown product formed. The suspension was chilled and stirred overnight. The solid product was then separated and washed by centrifugation and crystallized from freshly distilled tetrahydrofuran to which water was added to the cloudiness point. The product was again collected by centrifugation and dried over phosphorus pentoxide, yield 2.5 g (53.3%). The ^1H NMR spectrum of MPA in Me_2SO-d_6 at 250 MHz showed an approximate AB pattern centered at 8.03 ppm with $\delta_A - \delta_B = 0.10$ and $J_{AB} = 8$ Hz. Low-intensity satellites from the spin–spin coupling with ^{199}Hg ($J \approx 200$ Hz) could also be observed. The spectrum showed no impurities. The counterion attached directly to the mercury was partially replaced in some cases by azide ion, as evidenced by IR absorption, or by trifluoroacetate ion (^{19}F NMR). The ion could be restored by exchange with dilute acetic acid. When heated, the MPA salts decomposed at temperatures varying with the rate of heating. MPA in solution in Me_2SO shows a λ_{max} at 380 nm of 32 000 M^{-1} cm^{-1}, tailing off into the visible and falling to near zero at 300 nm.

Parvalbumin. Carp parvalbumin (p*I* 4.25) was prepared by the procedure of Pechere et al. (1971). Its identity and purity were established by $NaDodSO_4$–polyacrylamide gel electrophoresis and amino acid analysis. It was collected, dialyzed against 1.0 mM $CaCl_2$, lyophilized, and stored as a dry powder at −20 °C. It had $\epsilon_{254} = 2000\ M^{-1}\ cm^{-1}$ and ϵ_{280} near zero.

Preparation of Parvalbumin Modified with MFP. Ten milliliters of a 2.5 mM solution of MFP was applied to a 1 × 50 cm Sephadex G-25 column, followed by 10 mL of column buffer. A 1.25-mL sample of 2 mM parvalbumin containing 2 mM dithiothreitol was then applied and column buffer flow started. The parvalbumin, being excluded from the gels, moves rapidly away from the dithiothreitol, passes through the retarded reagent zone, and elutes with the void volume (25–40 mL). Since parvalbumin has $\epsilon_{254} = 2000\ M^{-1}\ cm^{-1}$ and $\epsilon_{280} \approx 0\ M^{-1}\ cm^{-1}$, while MFP has $\epsilon_{254} = 325\ M^{-1}\ cm^{-1}$ and $\epsilon_{280} = 2800\ M^{-1}\ cm^{-1}$, a 1:1 molar reaction product should have $A_{280}/A_{254} \approx (2800 + 0)/(2000 + 325)$ or 1.20. In several preparations, the eluate showed absorbance ratios very close to this figure, indicating 100% modification. This was confirmed by determining the amount of protein in solution by the Lowry method and determining the mercury content by using atomic absorption. The excess dithiothreitol elutes from the column as the second peak (55–70 mL) and is also fully loaded with MFP, as judged from the absorption at 280 nm. Unreacted excess reagent elutes after an additional column volume of buffer. The column is unaffected and may be reused.

Apoparvalbumin–MFP. Chelex resin was swollen and equilibrated by treatment with several washes of 10 mM Tris buffer, pH 7.5, and then filtered off. Parvalbumin–MFP (1 mM in Tris buffer, pH 7.5, and 100 mM KCl) was slurried with the resin (1 g of resin/5 mL of protein solution) for 45 min at 60 °C. The protein solution was recovered by filtration through a 8 × 1 cm disposable polypropylene column (Iso-lab), which had previously been washed exhaustively with Chelex-treated water, and was collected and handled in polypropylene ware. The calcium content of the solution was determined by atomic absorption and was less than 10^{-7} M, so that the protein was about 0.01% in the Ca^{2+} form.

Reactions with Glutathione. Derivatives of glutathione were prepared by mixing appropriate quantities of stock solutions of glutathione and the reagent at low pH to yield the desired stoichiometry.

NMR. Proton NMR spectra at 250 MHz and ^{19}F spectra at 235.2 MHz were obtained in the correlation mode on the MPC-HF spectrometer at the NMR Facility for Biomedical Studies, at Carnegie-Mellon University. ^{19}F spectra of 94.6 MHz were recorded in the Fourier transform (FT) mode at the Regional NMR Facility of the University of California,

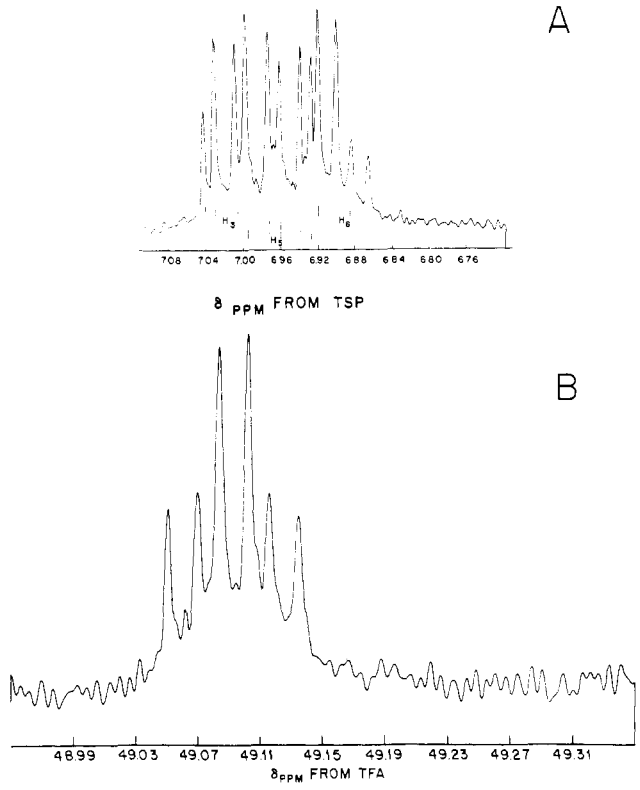

FIGURE 1: 250-MHz ^1H (A) and 235-MHz ^{19}F (B) spectra of MFP in ^2H_2O, pH* 2.3, aromatic region only.

Table I: NMR Parameters for MFP with Numbering As Shown

	free MFP 10 mM in ^2H_2O	1:1 MFP:GSH 10 mM in ^2H_2O, pH 2.2
δ [a][H(3)]	7.02	7.09
δ [H(5)]	6.97	6.93
δ [H(6)]	6.90	6.93
δ [b](F)	49.09	45.90
$J_{3,5}$ [c]	2.8	2.8
$J_{3,6}$	[d]	
$J_{3,F}$	8.5	8.5
$J_{5,6}$	9.0	9.0
$J_{5,F}$	9.0	9.0
$J_{6,F}$	4.5	4.5

[a] In ppm from TSP. [b] In ppm from TFA. [c] In hertz. [d] Unresolved.

San Diego. T_1's were determined in each case by the inversion–recovery method.

Results and Discussion

NMR Observation of MFP, Free and Combined. The proton and ^{19}F NMR spectra of aqueous MFP (Figure 1) are in accord with its structure, and the assignments have been confirmed by proton–proton and fluorine–proton decoupling experiments. The NMR parameters obtained are listed in Table I, for free MFP in aqueous solution at pH 2.2 (in the presence of trifluoroacetic acid to prevent hydrolysis) and for the 1:1 reaction product of MFP and glutathione, 10 mM in D_2O at pH 2.2. The titration of the phenolic hydroxyl of free MFP in water may be followed by monitoring the ^{19}F chemical shift which increases from 54.5 ppm at pH 2.2 to 62.0 ppm from TFA at pH 11. The titration curve indicates a pK_a of 9.5.

The effect of incomplete modification on the proton spectrum of glutathione is shown in Figure 2, which displays

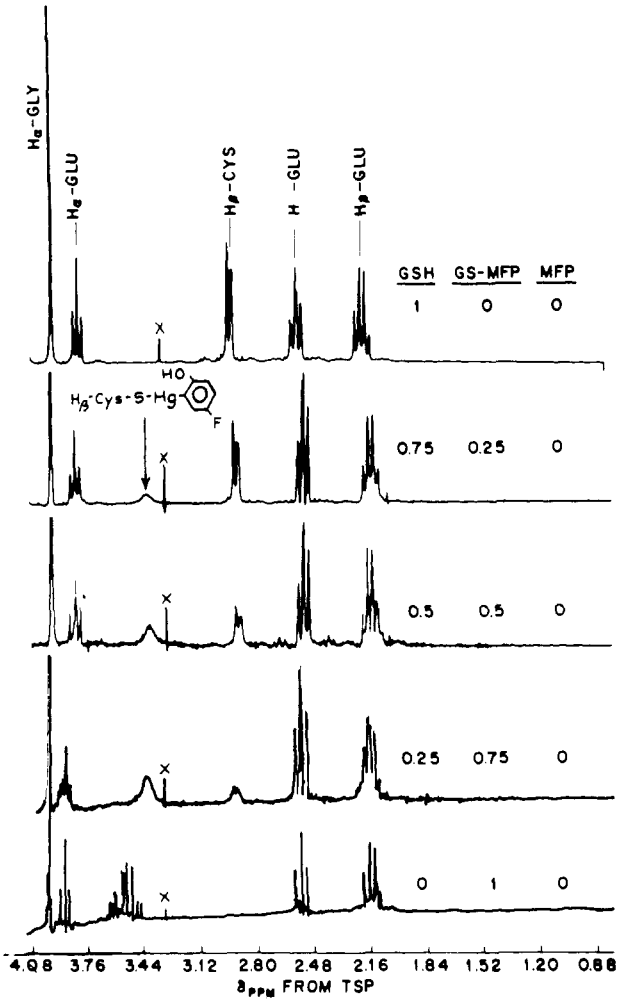

FIGURE 2: 250-MHz ^1H spectra of glutathione with 0, 0.25, 0.5, 0.75, and 1.0 equiv of MFP added, in ^2H_2O, pH* 2.30.

spectra of 4:1, 4:2, 4:3, and 4:4 reaction products of glutathione and MFP. The β protons of Cys-2 exhibit a downfield shift of 0.60 ppm on formation of the S–Hg bond. The appearance of the spectra of the partially reacted material is characteristic of an exchanging system, in which arylmercuri groups are transferred at a moderate rate between sulfhydryl groups. In partially modified glutathione, the H_β peaks corresponding to modified and unmodified cystines are broadened, indicating mutual exchange. This behavior has been noted previously in observations of the association of methylmercury with thiols (Simpson, 1961). Since the association constant is so high, it seems unlikely that this exchange occurs via first-order dissociation of the glutathione–MFP complex, but rather via species in which one arylmercury group is coordinated to two thiol groups. Thus, incomplete modification of proteins with MFP may give partially shifted or broadened lines from the protein moiety. The signals from the MFP itself, which is of course 100% reacted, are insensitive to the presence of excess unreacted glutathione (or protein).

NMR Measurements of MFP–Parvalbumin. MFP was attached to parvalbumin by using a gel exclusion chromatographic technique (see Experimental Procedures for details).

The proton spectrum of parvalbumin–MFP showed very little change from that of parvalbumin itself recorded under the same conditions, indicating that no gross structural changes had resulted from the attachment of the arylmercuri group.

The pK_a of the fluorophenolic group attached to parvalbumin was determined by titration, monitoring the ^{19}F resonance,

Table II: Calculated Longitudinal and Transverse Relaxation Parameters for Parvalbumin-MFP

^{19}F spectrometer frequency (MHz)	T_{1D} (s)	$T_{1,CSA}$ (s)	T_1 (s)	$1/T_{2D}$ (s^{-1})	$1/T_{2,CSA}$ (s^{-1})	$1/T_2$ (s^{-1})
94.6	0.28	2.17	0.25	9.5	15.6	25.1
235.2	0.56	1.54	0.41	8.75	96.5	105.2

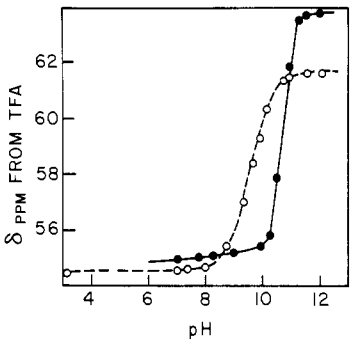

FIGURE 3: Titration curves for free MFP (open circles) and for MFP-modified parvalbumin (closed circles) (^{19}F chemical shift vs. pH), in ^2H_2O solvent.

and shown to be higher, 10.75, than in the free reagent (Figure 3). This suggests that the fluorophenol ring is held in a nonpolar environment in the complex. There is a slight change in the ^{19}F chemical shift over the region pH 7–9, suggesting the proximity of a group titrating in this range.

T_1 values for ^{19}F in free MFP and MFP–parvalbumin were determined by the inversion–recovery method using FT pulsed methods at 94.6 MHz. It was interesting to observe that the members of the ^{19}F multiplet from free MFP did not exhibit identical relaxation schedules, the signal at lowest field recovering more slowly than that at highest field. Such behavior is not unexpected (Werbelow & Grant, 1977) but not often seen as pronounced as here (Figure 4). The average T_1 for the multiplet was 3.0 ± 0.5 s. The T_1 for parvalbumin–MFP was 0.28 s at 94.6 MHz and 0.39 s at 235.2 MHz (Figure 5). No significant change in T_1 was observed for parvalbumin–MFP on changing the solvent from D_2O (T_1 = 0.28 s) to H_2O (T_1 = 0.26 s) at 94.6 MHz (Figure 6). All measurements were made on solutions 1 mM in parvalbumin–MFP, 50 mM in Tris buffer at pH 7.0, and 100 mM in KCl. It was also noted that the ^{19}F signal line width was 25 Hz at 94.6 MHz and 35 Hz at 235.2 MHz.

The apoprotein was prepared by treatment with Chelex resin in order to determine the effect of calcium removal on the spectral parameters; the measured T_1 at 95.6 MHz decreased to 0.20 s, and the line width of the ^{19}F signal increased to 50 Hz.

A possible interpretation of these results is that the fluorinated ring is held fairly tightly to the protein, perhaps lying in a cleft, and that the motion of the ring is that of the overall reorientational motion of the protein. Depolarized light scattering and ^{13}C relaxation studies (Opella et al., 1974) have shown this motion to be characterized by a rotational correlation time, τ_c, of 12 ns. The ^{19}F will undergo relaxation from two sources: dipolar interaction with the adjacent protons on the fluorophenol ring and interaction with the fluctuating field resulting from chemical shift anisotropy. Using the standard formulas for longitudinal and transverse relaxation rates, normal internuclear distances, and a chemical shift anisotropy of 109 ppm, based on analogy with fluorobenzene (Hull & Sykes, 1975), one calculates relaxation rates, as given in Table II.

The T_1 values agree surprisingly well with the experimentally determined ones. In terms of line widths, the calculated

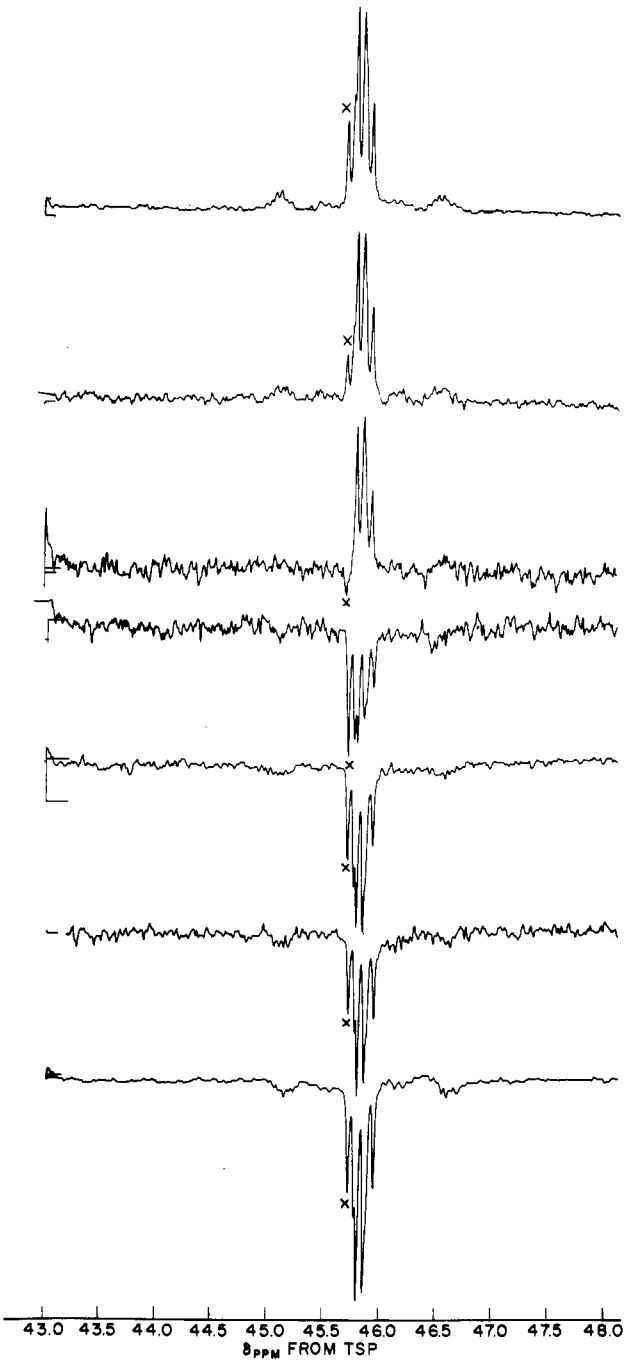

FIGURE 4: Inversion–recovery of the ^{19}F signal of MFP at 94 MHz illustrating unequal recovery rates of the different spin components.

values of $1/T_2$ predict line widths of 8 and 33 Hz at 94.6 and 235.2 MHz, respectively. Comparison with experiment is less easy here because of the multiplet nature of the signals which is unresolved in the parvalbumin–MFP signal. Qualitatively, the spectra appear to agree with the prediction.

The insensitivity of the T_1 relaxation time of ^{19}F to a change in solvent from H_2O (0.26 s) to D_2O (0.28 s) and the high pK_a are both consistent with a structure in which the fluorophenol ring is not exposed to solvent.

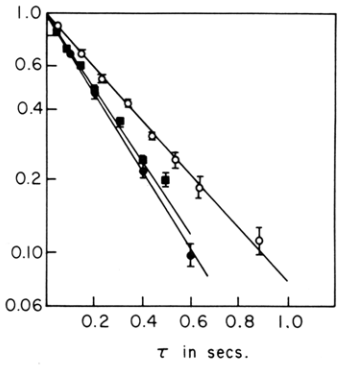

FIGURE 5: Semilog plots for the determination of T_1 for the ^{19}F signal of parvalbumin–MFP: in ^2H_2O at 235 MHz (○); in ^2H_2O at 94 MHz (■); in ^1H_2O at 94 MHz (●).

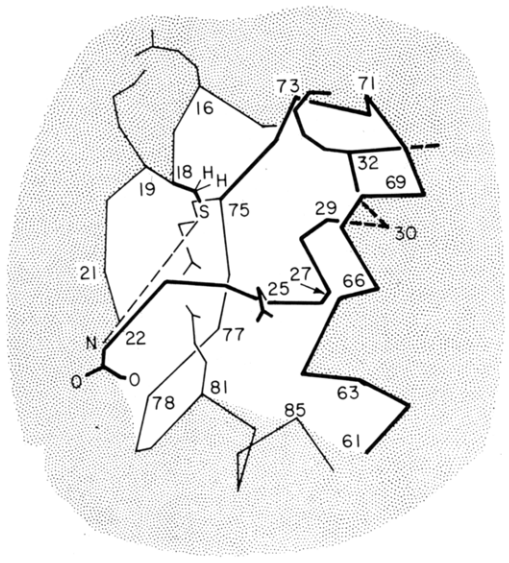

FIGURE 6: Detail of the structure of modified parvalbumin using the X-ray-determined atomic coordinates of Kretsinger and Nockolds. The dashed line attached to the S of Cys-18 represents the nitrene–phenylmercuri group, the nitrene being within the bonding radius of C_β of Asp-22.

After removal of calcium with Chelex, the ^{19}F signal shifts to higher fields, indicating that the ^{19}F is now more exposed to solvent (Roberts et al., 1977). The exposure to solvent protons also shortens its T_1 relaxation time to 0.20 s and causes increased line broadening. The latter may arise, in part, from incomplete chemical shift averaging. Thus, removal of Ca^{2+} causes a conformational change, that affects greater than 20 Å away the increased exposure of the fluorophenol ring to solvent, by the movement of the Cys SH to a solvent-accessible position. Donato & Martin (1974) have observed a faster rate of reaction of Ellman's reagent with parvalbumin upon calcium removal.

p-(Acetoxymercuri)phenyl Azide. The reagent is reasonably soluble in dimethyl sulfoxide. The proton spectrum of the solution consists of an apparent AB quartet with $J = 8.0$ Hz, $\delta_A = 7.98$, and $\delta_B = 8.14$. No extraneous peaks, other than the expected ^{199}Hg satellite peaks, were detected. Peptide and protein sulfhydryl groups may be modified conveniently by adding 1 equiv of reagent dissolved in dimethyl sulfoxide to an aqueous solution of the peptide or protein. Glutathione was quantitatively modified in this manner, as judged by the shift of the Cys CH_2 signal to $\delta = 3.38$ (vide supra). The azidophenyl protons in the product gave an AB quartet as before, with $J = 8.0$ Hz, unchanged, and $\delta_A = 7.41$ and $\delta_B = 8.45$, about 0.5 ppm upfield of the signals from the free reagent.

Photolysis. MPA (5 mM) in dimethyl sulfoxide was photolyzed in a quartz cuvette placed about 10 cm in front of an unfiltered high-pressure mercury arc, and the decomposition of the azide was followed by the disappearance of the azide band at 2140 cm^{-1}. The band intensity decreased rapidly to 50% in the first 5 min; however, a complex absorption began to grow in this region and after 10–15 min completely obscured the original MPA absorption. The same complex absorption band was produced on irradiation of the dimethyl sulfoxide alone. An aqueous solution of unmodified parvalbumin was irradiated under the same conditions. Comparison of the amino acid analysis of the parvalbumin before and after irradiation showed that phenylalanine was rapidly and selectively destroyed. It appeared necessary, therefore, to introduce a filter which would remove the shorter wavelength components of the irradiating UV, causing unwanted reactions in the dimethyl sulfoxide and aromatic amino acid residues. Dimethyl sulfoxide itself, which cuts off fairly sharply at 340 nm, served well. When a 1-cm thickness of dimethyl sulfoxide was interposed between the mercury arc and the sample cuvette, no decomposition of dimethyl sulfoxide or of parvalbumin in the sample cuvette could be detected after 20 min of irradiation.

Photolysis of Parvalbumin–MPA. Freshly prepared aqueous solutions of parvalbumin and parvalbumin–MPA were photolyzed for 20 min with a dimethyl sulfoxide filter and then subjected to amino acid analysis. Examination of the analytical results from several runs before and after irradiation indicated essential invariance in the ratio of the amounts of valine, leucine, and isoleucine in all cases. The analytical figures were therefore normalized to a constant amount of leucine (9.077 μmol). On comparing the figures for parvalbumin and parvalbumin–MPA, one noted one conspicuous change: a reduction of 0.717 ± 0.147 μmol of aspartic acid after photolysis of the parvalbumin–MPA, indicating that the nitrene intermediate had reacted substantially with an aspartic acid residue. Tables showing all amino acid analyses and statistics are available from the authors on request.

In order to determine whether an aspartic acid residue is appropriately positioned to react with the nitrene generated from parvalbumin–MPA, we examined molecular graphics displays of parvalbumin, courtesy of the biotechnology facility at the University of California, San Diego. The representation was based on the molecular coordinates given by Kretsinger & Nockolds (1973). Normal interatomic distances suggest a length of 8.77 Å for the S to N distance in the thiomercurinitrene grouping; accordingly, a search was made for aspartic acid residues located at approximately this distance from the Cys-18 thiol group, and accessible to it without excessive steric strain caused by compression of the mercuriphenyl moiety and peptide backbone. While Asp-10 and Asn-25 appear to be possible candidates, the former is 12 Å away while the latter is too close. Aspartic acid residue 22, however, fulfills the requirements, and its interaction with the nitrene requires that the ring lie in a cleft in the protein surface delineated on one side by residues 16–22, and on the other 61–73. A slight rotation about the $C_{\alpha\text{-}21}$–$C_{\alpha\text{-}22}$ bond allows the nitrene (dashed line Figure 6) to approach within 0.6 Å of $C_{\beta\text{-}22}$, while maintaining the Asp-22 α-NH–Glu-81 ϵ-CO_2^- hydrogen bond. This in turn allows the important Arg-75–Glu-81 internal salt bridge to be maintained, a condition critical for parvalbumin's native tertiary structure.

Conclusions

Bifunctional arylmercuri reagents are convenient selective modifiers for thiol groups in peptides and proteins. The reagents may be tailored for the particular application in-

tended: a fluorine labeling reagent for NMR studies and an azide reagent for photolysis studies have been attached to parvalbumin. The results indicate that the groups are selectively attached to cysteine-18 and that the aryl rings lie in a cleft formed by residues 16–22 and 61–73 of parvalbumin.

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Registry No. MFP, 84332-82-1; MPA, 84332-83-2; *p*-fluorophenol, 371-41-5; mercuric trifluoroacetate, 13257-51-7; *p*-(acetoxymercuri)aniline, 6283-24-5; *S*-[(2-hydroxy-5-fluorophenyl)mercuri]glutathione, 84332-84-3; *S*-[(4-azidophenyl)mercuri]glutathione, 84332-85-4.

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Direct Enzyme-Catalyzed Reduction of Anthracyclines by Reduced Nicotinamide Adenine Dinucleotide†

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ABSTRACT: The *Vibrio harveyi* NADH:flavin oxidoreductase forms ternary complexes with NADH and daunomycin and with NADH and aclacinomycin A. The anthracyclines occupy the flavin binding site of this enzyme. Within this complex, the enzyme affects the catalytic reduction of the anthracycline by NADH. Under aerobic conditions, the reduced anthracyclines are rapidly oxidized by molecular oxygen and are cycled catalytically. Under anaerobic conditions, reductive elimination of the glycoside occurs for both. For daunomycin, the only product obtained is 7-deoxydaunomycinone, showing the stereospecific incorporation of a solvent proton at C-7. Given the redox chemistry of NADH and the stereochemistry of this protonation, the suggested mechanism for this conversion is synchronous 2e, H^+ (hydride) transfer to provide the daunomycin hydroquinone that reductively eliminates to the quinone methide. Solvent proton (electrophile) trapping of the quinone methide occurs while the quinone methide remains enzyme bound. In contrast, enzymatic reduction of aclacinomycin provides two products, 7-deoxyaklavinone (28%) and the 7-deoxyaklavinone dimer (67%), identical with the dimer observed previously from chemical and enzymatic aclacinomycin reduction. This product composition indicates that aclacinomycin comproportionation successfully competes against a hydroquinone elimination pathway. The mechanism for aclacinomycin glycoside elimination is probably comproportionation to a semiquinone molecular complex and then glycoside loss followed by collapse of the semiquinone methide molecular complex to the dimer. It is not possible to trap the quinone methide from daunomycin (by thiol or thiolate nucleophiles) nor the radical pair from aclacinomycin (by thiol hydrogen atom donors). The data provide the following conclusions. Most importantly, it has been established that the anaerobic redox chemistries of daunomycin and aclacinomycin are significantly different, in that different pathways are followed regardless of whether a hydride or one-electron reductant is used. For daunomycin, a hydroquinone reductive elimination has been proven feasible, but the resulting quinone methide is either inaccessible or (most probably) unreactive to solution nucleophiles. For aclacinomycin, a semiquinone reductive elimination dominates but undergoes preferential dimerization within a molecular complex rather than hydrogen atom abstraction from solution donors. These studies suggest that in the absence of a specific target, the reductive elimination pathways of these two anthracyclines (although different) are chemically innocuous.

The anthracycline antitumor antibiotics (Arcamone, 1981; Young et al., 1981) are among several antitumor antibiotics that are activated in vivo by electron transfer. During reductive activation, the quinone functional group of the anthracyclines accepts either one or two electrons (depending on the reducing agent) to provide semiquinone or hydroquinone reduced states, respectively. The relationship of these reduced anthracycline redox states to the potent biological effects these antibiotics express is the focus of much current research. On the basis of in vitro study, several immediate consequences of anthracycline redox turnover have been identified; these include the inhibition of enzymes, the covalent labeling of macromolecules, and the initiation of oxygen-dependent lipid peroxidation and DNA degradation pathways (Bachur et al., 1979; Pan et al., 1981; Sinha & Gregory, 1981; Oki, 1977;

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