Binding of Acrylonitrile to Parvalbumin

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Received April 24, 1995; accepted August 22, 1995

A previous study has shown that acrylonitrile (ACN) has a long half-life in rainbow trout muscle and that \[^{14}\text{C}]\text{ACN}\) appears to be bound to a 10,000 Da protein in muscle. The labeled protein was purified from muscle of trout exposed to \[^{14}\text{C}]\text{ACN}\), separated on 20% SDS–PAGE, and digested for amino acid analysis and sequence analysis. These studies indicated that the labeled protein was the Ca\(^{2+}\)-binding protein parvalbumin. Parvalbumin is an important calcium-binding protein thought to be involved in the regulation of calcium levels in various parts of the body ranging from neurons to fast-twitch muscle contractions. To study the reaction between parvalbumin and \[^{14}\text{C}]\text{ACN}\), frog parvalbumin was incubated with \[^{14}\text{C}]\text{ACN}\) in vitro under various conditions. These studies indicated that the maximum labeling occurred at 1 nmol/nmol parvalbumin and at pH 7. Amino acid analysis of the labeled protein indicated that the labeled amino acid was probably histidine, and endoproteinase Glu-C (V-8) digestion studies revealed that the \[^{14}\text{C}]\) was in the 1–81 amino acid segment of the protein, an area that contains two histidines.

Acrylonitrile (ACN) is an important industrial chemical which is used in the production of plastic, synthetic fibers, pharmaceuticals, dyes, and insecticides. ACN has been shown to produce a variety of toxicological effects, ranging from symptoms of cyanide poisoning acutely (Tanii and Hashimoto, 1984) to tumor formation in chronic studies. Acrylonitrile has been studied for carcinogenicity in rats (Maltoni et al., 1977; Bigner et al., 1985), and investigations have demonstrated a relationship between exposure to acrylonitrile and induction of tumors at several sites in the rat. ACN undergoes extensive metabolism via two major routes — glutathione conjugation and epoxidation. It has been postulated that metabolism to cyanoethylene oxide may mediate the carcinogenic and some of the toxic actions of ACN (Fennell et al., 1991a; Guengerich et al., 1981; Kopeky et al., 1980; Linhart et al., 1988; Ahmed and Patel, 1981). In addition, release of cyanide has also been shown to contribute to the toxicity of ACN. Although a considerable amount of research has been carried out on the metabolism and disposition of ACN in mammalian species, little work has been done to investigate the covalent binding sites as seen in various disposition studies. Although acrylonitrile has been shown to bind to liver microsomes (Peter and Bolt, 1981) and to hemoglobin (Fennell et al., 1991b), this binding is not thought to be involved in the toxicity of acrylonitrile. On the other hand, the toxicity related to covalent binding of acrylonitrile to these and other minor sites, such as muscle tissue, has not been studied in detail (Sato, 1981).

In a dispositional study of \[^{14}\text{C}]\text{acrylonitrile}\) in rainbow trout, it was found that the \(^{14}\text{C}\) had a relatively long half-life (110 hr) and much of the \(^{14}\text{C}\) was localized in muscle tissue, as shown by autoradiography, and bound to a protein of approximately 10,000 Da (Lech et al., 1995). The current study identifies this protein as parvalbumin as well as the conditions for binding in vitro. With the in vitro conditions optimized the amino acid involved and the location of binding within the protein could be investigated.

Parvalbumin has been shown to be involved in Ca\(^{2+}\) sequestration in fast-twitch muscles, in Ca\(^{2+}\) homeostasis in neurons at a variety of sites (Solbach and Celio, 1991), and has been implicated in regulation of gene expression (Cheema et al., 1991).

The disruption of calcium levels, in general, and levels of parvalbumin, in particular, have been associated with muscular dystrophy (Klug et al., 1985; Bertorini et al., 1982) and with Alzheimer’s disease (Ichimiya et al., 1989) and amyotrophic lateral sclerosis (Alexianu et al., 1994).

MATERIALS AND METHODS

\[^{1.2-14}\text{C}]\text{acrylonitrile}, SA 5.3 mci/mmol was obtained from Sigma Radiochemicals, St. Louis, Missouri. Frog and rabbit parvalbumin, parvalbumin antibodies, and bovine serum albumin (BSA) were obtained from Sigma Chemical Company. The trout hemoglobin was collected on site from untreated control rainbow trout. SDS–PAGE electrophoresis materials were obtained from Bio-Rad Laboratories, Richmond, California. All other chemicals were of the highest purity and were obtained from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin.

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Exposure of fish to $[^{14}C]$acrylonitrile. Rainbow trout (150 g) were exposed, in a static tank system, to $[2.3,^{14}C]$acrylonitrile at 1.002 μCi/fish (SA = 5.3 mCi/mmol). After 24 hr, the fish were terminated and the muscle was removed. The muscle was then homogenized 1:3 w/v in 0.1 M KCl buffer and the homogenate was centrifuged at 2900g for 20 min. The supernate was then aliquoted and stored at −20°C. The supernate was later centrifuged and used for SDS–PAGE, amino acid composition, and peptide sequence analysis.

Protein isolation, amino acid composition, sequence analysis, and Western blot immunoactivity analysis. Samples from the labeled muscle extracts were separated on 20% polyacrylamide gels. The samples were then electrobotted to polyvinylidene fluoride membranes (PVDF; Immunoblot, P, Millipore).

Briefly, samples were separated on 20% SDS Laemmli gels, 100 × 80 × 10.0-mm gels, and electrobotted for 2 hr at 100 V, 150 mA using a Mini Protein 2 (Bio-Rad) blotting chamber. The blotting buffer used was 0.025 M N-ethylmorpholine formate (pH 8.3)/methanol (10:1, v/v). $^{14}C$-labeled bands were detected using a phosphorimaging system. Membranes were stained with 0.2% amido black in methanol/water/acetic acid (1:1:0.15, v/v).

The protein samples were blotted and recovered from charge-modified PVDF membranes (Immobilon CD, Millipore) or nitrocellulose membrane (Micron Separations). Radioactive bands were cut out from the PVDF samples and eluted twice in 250 μl 70% formic acid. The two extractions were combined for digestion with cyanogen bromide. Briefly, two digestions were performed with 24 and 33 μg of labeled material, respectively. A 200-fold excess of cyanogen bromide based on cysteine residues was added in two additions 24 hr apart and the digest was performed for 48 hr at room temperature, in the dark, under nitrogen. To stop the digestions the samples were diluted with water and evaporated in a vacuum concentrator. The first digestion product was washed twice with 200 μl distilled water, evaporated, dissolved in Laemmli sample buffer, and applied to a SDS–PAGE gradient gel. The electroblotted fragments were stained with amido black as described previously.

Protein and peptide bands were excised for either amino acid composition analysis or N-terminal sequence analysis. Amino acid analysis was performed after acid hydrolysis in 5.7 N HCl/0.02% β-mercaptoethanol under vacuum for 20 hr at 110°C on a Beckman 6300 amino acid analyzer. Some amino acid analysis samples were performic acid oxidized to determine the cysteine content of the protein. Performic acid oxidation is performed by addition of 0.02 M HClO4 containing 0.01 M H2O2 and 0.01 M NaOH, pH 7.7 to 8.0, to pool samples and incubating at 55°C for 2 hr. The samples were then washed with water and evaporated in a vacuum concentrator.

The second digest was concentrated, redissolved in 8 M urea, and analyzed on a high-performance liquid chromatography (HPLC) system to collect peptide samples for further analysis. The cyanogen bromide fragments were separated on a Hewlett–Packard (Model 1090) HPLC system using a C-18 reverse-phase column. The HPLC buffers were as follows: buffer A consisted of 0.1% trifluoroacetic acid (TFA) in water (v/v), and buffer B consisted of 0.1% TFA in acetonitrile (v/v). The system was run as a gradient of 5% buffer A to 60% buffer B over 30 min.

Nitrocellulose membranes were subjected to Western blot analysis. Western blots were developed using a 1/1000 dilution of a monoclonal antibody specific to calcium parvalbumin as the primary antibody, a 1/2500 dilution of Bio-Rad goat anti-mouse IgG horseradish peroxidase conjugate for the secondary antibody, and the (Sigma) DAB development reaction was used to develop the blot. The membrane, if radioactively labeled, was then subjected, qualitatively, to either the Phosphor Imager (Molecular Dynamics, Inc.) for 72 hr or applied to X-ray film (Kodak) for 21 days at −80°C.

In vitro studies. After establishing that the $^{14}C$-labeled protein produced in vivo was parvalbumin, studies were carried out to determine if parvalbumin could be $^{14}C$-labeled in vitro and the conditions under which such labeling would occur. Parvalbumin from frog muscle was used in the in vitro studies because it is functionally the same as trout parvalbumin, has a similar amino acid composition, and is easily attainable.

The procedure following the various incubations that will be described in the next few sections are as follows: sample reactions were stopped with 100 μl of a 10 mg/ml BSA/H2O solution and vortexed. To precipitate the protein, 4.5 ml of a 12% TCA (v/v) solution was added. The solution was then placed on ice for 20 min to aid the precipitation of the protein. Samples were then subjected to vacuum filtration through a 0.7 μm PFG5 Millipore filter. The protein, still bound to the filter, was then washed three times with 10 ml of 66 mm Tris–HCl buffer (pH 7.4). The filters were then transferred to scintillation vials for counting on a Packard scintillation counting system.

Temperature and buffer effects. Parvalbumin from frog muscle was incubated in three different buffers. The buffers were chosen to give the reaction a variety of ionic and pH conditions. Each buffer was run at two different temperatures, and 2.5 nmol of $[^{14}C]$ACN was added to each reaction mixture. The reactions were incubated for 24 hr.

The buffers were as follows. 100 mm KCl (pH 5.4), 66 mm Tris–HCl (pH 7.4), and 10 mm phosphate buffer (pH 6.9). The two temperatures were 21 (ambient) and 37°C.

Time course of labeling. Parvalbumin from frog muscle was incubated in 10 mM phosphate buffer with 2.5 nmol of $[^{14}C]$ACN added to each reaction mixture. The incubation was run as a time course experiment to determine the linearity of the reaction. The study was run at 37°C for a total of 24 hr.

Staphylococcal protease (V-8) digestion method. Parvalbumin from frog muscle was incubated with acrylonitrile as previous stated for 24 hr. The excess acrylonitrile was dried off by vacuum filtration. The V-8 protease (Sigma Chemical) was added to the labeled parvalbumin in a ratio of 0.02/1 enzyme to protein. The digestion was run in both dialyzed and undialyzed forms at 37°C for 48 hr at pH 7.8 in 10 mM phosphate buffer after which the products were run on a 20% SDS gel and blotted to PVDF membrane, as stated previously, for further analysis.

Multiple protein study. Various proteins, including frog and rabbit parvalbumin, BSA, and trout hemoglobin, were incubated in 10 mM phosphate buffer with 2.5 nmol of $[^{14}C]$ACN added to each reaction mixture. The incubations were run to determine the selectivity of ACN for parvalbumin.

Parvalbumin protein dependence. Varying amounts of parvalbumin from frog muscle tissue were incubated in 10 mM sodium phosphate buffer with 2.5 nmol of $[^{14}C]$ACN added to each reaction mixture. The incubations were run to determine the selectivity of ACN for parvalbumin.

Acrylonitrile dependence. Parvalbumin from frog muscle tissue was incubated in 10 mM sodium phosphate buffer with 2.5 nmol of $[^{14}C]$-labeled acrylonitrile at 37°C for 24 hr. The parvalbumin was added to the incubation mixture in a range from 0 (control) to 12 μg of protein.

Calcium dependence. Parvalbumin from frog muscle tissue was incubated in 10 mM sodium phosphate buffer with varying amounts of $[^{14}C]$-labeled acrylonitrile added to each reaction mixture. The study was run at 37°C for 24 hr. The purpose of this study was to check if the calcium-bound structure of the protein was needed to label the protein or if the calcium-free form would also bind ACN.

The frog parvalbumin was run in both dialyzed and undialyzed forms. Following incubation with 50 mM EGTA to remove the bound calcium, dialysis was performed, in 8-kDa molecular weight cutoff dialysis tubing, versus 5 liters of sterile MilliQ water overnight at 4°C with constant stirring. The samples were then incubated as stated in the other studies.

pH dependence. Parvalbumin from frog muscle was incubated at various pH ranging from pH 5 to pH 9, of 10 mM phosphate buffer with 2.5 nmol of $[^{14}C]$-labeled acrylonitrile added to each reaction mixture. The study was run at 37°C for 24 hr. The pH was tested to see the effects of varying the pH on the binding of the compound to the protein.
TABLE 1

Amino Acid Composition of the [14C]Acrylonitrile-Labeled Bands

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>12-kDa band (actual)</th>
<th>10-kDa band (actual)</th>
<th>Sequenced 10-kDa* (actual)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>6</td>
<td>6</td>
<td>nd</td>
</tr>
<tr>
<td>Aspartate</td>
<td>11</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Threonine</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Serine</td>
<td>10</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Glutamate</td>
<td>11</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Proline</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Glycine</td>
<td>19</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Alanine</td>
<td>13</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Valine</td>
<td>4</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Methionine</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Leucine</td>
<td>7</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Histidine</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lysine</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Arginine</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

* PVDF strips were hydrolyzed after initial sequence attempt.

The calculation is based on a total number of 108 amino acids as reported in most of the related parvalbumins.

RESULTS

Amino Acid Composition and Sequences of 14C-Labeled Proteins

Two 14C-labeled bands of the approximate molecular weight of 10 to 12 kDa were detected after gel electrobloptting. Table 1 shows the amino acid composition of these bands. A comparison of the amino acid compositions of the two radioactive bands shows the close relation of these two proteins to each other, but also indicates some differences. The difference in the number of alanine, leucine, tyrosine, and phenylalanine residues points to the fact that the proteins are indeed similar but different.

Both bands could be resolved by 20% SDS-PAGE but migrated relatively close to each other. The question of possible cross-contamination of the two bands was resolved by cyanogen bromide (CNBr) digestion analysis. Fragments were generated from the 10-kDa and not from the 12-kDa band indicating the presence of methionine in the lower band only, which was verified by the previously mentioned amino acid analysis. The sequence data, after CNBr digestion, of the 10-kDa protein gave the following sequence: I-G-I-X(D)-E-F-A-X-L-V.

A list of the top three homology search matches, for the 10-kDa band, is shown in Table 2. These sequences were obtained using the Genetics Computer Group, Inc. (GCG) sequence homology search program. The results show that the unknown trout digestion fragment was part of the parvalbumin protein. These data indicate that the protein labeled with [14C]ACN is in all probability parvalbumin, an important calcium-binding protein in muscle and neuronal tissue.

Because the sequence for rainbow trout parvalbumin has not been published, it was not possible to attempt a full sequence match or to differentiate between the alpha or beta forms. The sequence of frog parvalbumin (alpha) is a close match to many published parvalbumins as well as being commercially available.

Figure 1 shows the results of SDS-PAGE of samples of frog parvalbumin incubated at room temperature and at 37°C.

![Figure 1](image-url)

FIG. 1. SDS-PAGE of frog parvalbumin. Amido black stain. (A) Molecular weight ladder, (B and D) contain β-mercaptoethanol in the sample buffer, (C and E) do not contain β-mercaptoethanol. (B and C) run at 25°C, (D and E) run at 37°C.
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with and without the presence of β-mercaptoethanol (BME) in the sample buffer. It can be seen (Fig. 1) that parvalbumin exists as a dimer with a MW of approximately 26,600 Da. When incubated in the presence of BME at either room temperature or 37°C, the protein MW shifts to the 12,000 area, indicating the presence of the monomeric form produced by the reduction of disulfide bonds. The presence of the higher-molecular weight bands is thought to be due to a polymeric form of parvalbumin through either disulfide bond or salt bridge formation.

Figure 2 shows the time course of parvalbumin labeling with [14C]ACN, the lag period for labeling, and relatively long time scale, which is in hours. The lag period is possibly due to the presence of the antioxidant, p-methoxyphenol, in the acrylonitrile solution.

Figures 3 and 4 depict the dependence of labeling of frog parvalbumin by [14C]ACN on the ACN and parvalbumin concentration. Both curves have a linear portion and show a tendency toward a labeling saturation at higher concentrations of ACN and parvalbumin. The presence of radioactivity on the filters without parvalbumin is thought to be due to a low-level affinity by acrylonitrile for the filter. Multiple filter types were tested (data not shown) for this study, with the 0.7-μm PFG5 Millipore filter having the lowest overall affinity for the straight acrylonitrile.

The effect of pH on parvalbumin labeling by [14C]ACN is shown in Fig. 5. Incorporation of 14C was minimal at pH 9, intermediate at pH 5, and highest at pH 7. Assuming that the pH changes did not affect the degradation of the adduct, this finding indicates that the labeled region or regions of the protein may contain a selectively labeled amino acid due to the region's higher selectivity with ACN at pH 7 when compared to other regions of the protein. The fall in labeling at pH 9 was unexpected because the charge on most electrophilic amino acids would be the same at pH 7 as at pH 9. This may be caused by a pH effect on the structure of...
parvalbumin, hence decreasing the binding of acrylonitrile or the inherent instability of the bound acrylonitrile at alkaline pH.

Figure 6 illustrates the comparison of rates of labeling of several proteins by $[^{14}\text{C}]\text{ACN}$. The data indicate that while hemoglobin and BSA can also be labeled, the two types of parvalbumin appear to be more readily adducted than the former.

When $\text{Ca}^{2+}$ is removed from parvalbumin by prolonged dialysis against EGTA and incubated with $[^{14}\text{C}]\text{ACN}$, there is little or no labeling of the protein (Table 3). Addition of $\text{Ca}^{2+}$ in a preincubation of undialyzed parvalbumin restores the ability of $[^{14}\text{C}]\text{ACN}$ to label the protein.

**Site of Covalent Binding in Parvalbumin**

Through the use of Western blotting of *in vitro*-labeled muscle extracts it was shown that acrylonitrile labels both the monomer and the dimerized forms of parvalbumin.

<table>
<thead>
<tr>
<th>Protein form</th>
<th>mmol of acrylonitrile incorporated per nanomole of parvalbumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parvalbumin plus $\text{Ca}^{2+}$</td>
<td>0.1730</td>
</tr>
<tr>
<td>Parvalbumin minus $\text{Ca}^{2+}$</td>
<td>0.0084</td>
</tr>
<tr>
<td>Control (no parvalbumin)</td>
<td>0.0047</td>
</tr>
</tbody>
</table>

* Parvalbumin containing 2 mol $\text{Ca}^{2+}$/mole based on assay.

**DISCUSSION**

In the previous studies with trout, the half-life of ACN was prolonged due to the interaction with parvalbumin (Lech et al. 1988).

Evidence for a possible binding site was provided through performic acid oxidation and hydrolysis of an *in vitro*-labeled quantity of parvalbumin and unlabeled parvalbumin. Both preparations were subjected to amino acid composition analysis and the results compared. The labeled sample showed a loss of histidine residues, while the histidine residues were retained when the unlabeled parvalbumin was used for comparison (Table 4).

<table>
<thead>
<tr>
<th>Labeled parvalbumin</th>
<th>Unlabeled parvalbumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>1</td>
</tr>
<tr>
<td>Cysteic acid</td>
<td>2</td>
</tr>
<tr>
<td>Histidine</td>
<td>0</td>
</tr>
<tr>
<td>Lysine</td>
<td>14</td>
</tr>
<tr>
<td>Serine</td>
<td>6</td>
</tr>
<tr>
<td>Threonine</td>
<td>5</td>
</tr>
</tbody>
</table>

* Parvalbumin was labeled with $[^{14}\text{C}]\text{ACN}$ as described under Materials and Methods.

Following the amino acid composition analysis, the *in vitro*-labeled protein was subjected to V-8 protein digestion. The digestion products were run on a SDS–PAGE gel and blotted to PVDF membrane. The amido black-stained membrane showed several distinct bands. An autoradiographic analysis revealed a radioactive band of approximately 8000 Da which was subjected to amino acid sequence analysis. The sequence analysis of the 8000-Da V-8 digestion product showed that the labeled band was the N-terminal end of the protein. Because V-8 cleaved the glutamate at position 81, the ACN label appears to be present within the first 80 amino acids of the protein (Table 5). It is within this part of the protein that the two histidine residues reside.

**TABLE 3**

**Effect of $\text{Ca}^{2+}$ on Labeling of Parvalbumin by $[^{14}\text{C}]\text{ACN}$**

**TABLE 4**

**Labeled and Unlabeled Parvalbumin after Performic Acid Oxidation and Hydrolysis**

**TABLE 5**

**V-8, $[^{14}\text{C}]$-Labeled Digestion Fragment Sequence Match with Known, N-Terminal Sequence in Frog Parvalbumin (p18087)**

<table>
<thead>
<tr>
<th>Parvalbumin fragment</th>
<th>M-H-M-T-D-L-L-X-A-G</th>
<th>% Identity with GCG</th>
</tr>
</thead>
</table>

* | a perfect match; : , a similar functional match for the amino acid listed.
et al., 1995). In various mammalian species, there is covalent binding of ACN to unknown sites, possibly proteins. The binding appears to incorporate only a small portion of the dose; however, from a toxicological viewpoint the unidentified binding sites could be involved in the multiple toxic actions of ACN.

In addition to elucidating the nature of the protein to which ACN is bound in fish muscle, the studies described in this report have defined conditions and factors regarding the labeling of frog parvalbumin. The in vitro studies themselves indicate that an impurity or a metabolite is probably not involved in the in vivo binding of ACN. The nature of the chemical reaction involved in binding is not precisely known; however, participation of free radicals in the binding is suggested by the lag period seen in the labeling time course and the presence of an antioxidant in the [14C]ACN. The antioxidant, p-methoxyphenol (p-MOP), is present to suppress any free radical formation of ACN, hence slowing down the mechanism leading to polymerization. In the reaction incubations the concentration of p-MOP is much lower than that in the supplier's stock solution which could possibly lead to formation of free radicals by ACN over a shorter period of time. The answer to this question awaits isolation and structural analysis of the adduct.

Several lines of evidence indicate that histidine residues may be a site for labeling with [14C]ACN. The most interesting of these is the loss of the histidine residues, in treated parvalbumin, on performic acid oxidation analysis/amino acid composition. This shows, in all likelihood, that histidine is the adducted site. The digestion of the labeled parvalbumin with V-8 showed that the labeled amino acid(s) was in the first 80 residues of the protein. The histidine residues are located at positions 2 and 27, hence lending support to the idea that a histidine is the site of ACN binding. Further evidence for histidine being the labeled site is the interference that Tris buffers have on the binding (data not shown). Phosphate buffer allowed for more labeling than the Tris buffer at similar pH (data not shown). This could be due to the presence of an amino group in the Tris competing with the amino group on the histidine side chain, hence decreasing the reactivity of acrylonitrile with the protein. Moreover, alkaline pHs have been shown to be optimal for the reaction of acrylonitrile with other basic amino acids such as lysine (Friedman, 1967; Cavins and Friedman, 1968). The mechanism of histidine labeling with ACN awaits the isolation of the amino acid adduct and its positive identification.

One of the more interesting observations in this study is the inability to label parvalbumin with acrylonitrile when it is in the Ca2+-free form. This is similar to the finding that certain antibodies do not react with Ca2+-free parvalbumin, but react strongly when it is in the Ca2+-loaded form. The latter is thought to be due to a significant conformational change in the protein caused by the removal of Ca2+ (Tinner et al., 1990).

From a toxicological point of view, if the binding of ACN to parvalbumin causes conformational changes in the protein which affect calcium binding, then cellular function of parvalbumin may be affected. The underlying hypothesis concerning the interaction of ACN with parvalbumin concerns an ACN-induced change in conformation of parvalbumin leading to changes in calcium binding by the protein. Preliminary data indicate that ACN binding to parvalbumin has an influence on the conformation of parvalbumin based on the luminescence of the phenylalanine residues (Permyakov et al., 1977).

ACKNOWLEDGMENTS

This research was supported by Cytec Inc. and Grant F49620-94-1-0264 from the Air Force Office of Scientific Research. We also thank Brady Stoner for his excellent technical assistance.

REFERENCES


