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Isolation of Neuronal Parvalbumin by High-Performance Liquid Chromatography. Characterization and Comparison with Muscle Parvalbumin†

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ABSTRACT: Neuronal parvalbumin has been isolated from rat brain and purified to homogeneity by high-performance liquid chromatography (HPLC) on reverse-phase supports. This procedure includes four consecutive chromatographic steps with an overall protein recovery of 74% and a 26 400-fold purification. The concentration of parvalbumin was found to be approximately 10 mg/kg wet weight in brain tissue, which is about 100 times lower than that in rat muscle. The physical properties of brain parvalbumin are described and compared with those of the muscle counterpart. These proteins were identical in their molecular weights (12 000), isoelectric points (4.9), retention times on C-18 reverse-phase HPLC columns, Ca^{2+} content (two per molecule), amino acid compositions, and immunological properties. A comparison of the tryptic peptide maps of brain and muscle parvalbumin by analytical HPLC also revealed identity and showed that the isolation method described here did not alter the chemical structure of the protein.

The role of calcium in cellular regulation is thought to be mediated by Ca^{2+} receptors such as calmodulin, troponin C, S-100 protein, vitamin D dependent Ca^{2+}-binding protein (CaBP),[1] and parvalbumin. Their Ca^{2+}-binding sites display significant internal structural homology, a fact that has suggested evolution from a smaller ancestral precursor by gene duplication [for reviews, see Kretsinger (1980) and Siegel et al. (1980)]. Whereas calmodulin is involved in regulating a broad spectrum of cellular activities and troponin in regulating muscle contraction, the physiological role(s) of S-100, vitamin D dependent CaBP, and parvalbumin are less clear. The physical properties of muscle parvalbumins have been mainly described for lower vertebrates and more recently for mammals [for reviews, see Pechère et al. (1973), Hamoir (1974), and Kretsinger (1980)]. When antibodies against rat muscle parvalbumin are used, immunological cross-reactivity has only been detected in a few organs, including the brain from lower vertebrates (Gosselin-Rey et al., 1978; Gerday et al., 1979), mammals (Baron et al., 1975), and chicken (Heizmann & Strehler, 1979). Using a monospecific antiserum against rat muscle parvalbumin, Celio & Heizmann (1981) found that the immunologically active material in rat brains is restricted to a distinct subpopulation of neurons.

Characterization of the immunological active components present in brain preparations has not been attempted probably because of the small quantitites present, e.g., estimated to be as low as 2 mg/kg of rabbit brain (Baron et al., 1975). Due to the usual poor overall yields experienced with the more "conventional" isolation methods (ion exchange, gel filtration, etc.), quite large amounts of starting material would be required for the preparation of milligram quantities of such proteins. Based on our previous observations concerning the chromatographic behavior of peptides (Hughes et al., 1979; Wilson et al., 1981a) and proteins (Wilson et al., 1982a,b) on reverse-phase supports, we felt that such methodology might well offer the possibility to prepare enough of the material to carry out a comparative study with muscle parvalbumin. The isolation of rat brain parvalbumin by high-performance liquid chromatography (HPLC) is described, and the amino acid analysis, two-dimensional gel pattern, and Ca^{2+} content as well as the immunological properties are compared with those of the muscle counterpart. In addition, HPLC, a sensitive and efficient tool to carry out peptide analysis in the subnanomole range (Wilson et al., 1981b), was chosen to prove the similarity (if not identity) of both proteins.

Materials and Methods

Proteins and Chemicals. Parvalbumin from leg muscles of 60-day-old rats (SIV-50) was prepared as described for the chicken protein (Strehler et al., 1977). The single alteration in the procedure was the addition of the following protease inhibitors to the homogenization medium: pepstatin (1 μM), PMSF (0.4 mM), TPCK (0.15 mM), leupeptin (1 μM), all

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[1] Abbreviations: HPLC, high-performance liquid chromatography; CaBP, vitamin D dependent Ca^{2+}-binding protein; PMSF, phenylmethanesulfonyl fluoride; TPCK, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; troponin C, Ca^{2+}-binding subunit of troponin; EDTA, ethylenediaminetetraacetate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetate; Tris, tris(hydroxymethyl)aminomethane.

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from Sigma; Trasylol (30 units/mL) from Bayer. TPCK-trypsin was from Worthington; EGTA and EDTA were from Fluka. Calmodulin and S-100 protein from bovine brain, troponin C from rabbit muscle, and Ca^{2+}-binding proteins from sandworm and crayfish were obtained from Dr. J. Cox (Biochemistry Department, University of Geneva, Switzerland).

HPLC Techniques. The HPLC system consisted of two Altex Model 110 pumps, a Rheodyne Model 7125 injector (600-μL sample loop), a Kontron Model 200 microprocessor, and an Uvikon 725 spectrophotometer with an 8-μL flow-through cell for UV measurements at 220 nm. Samples were loaded either by using the injector valve or directly by using the pump normally used for the A buffer. The following reverse-phase supports were utilized: (1) LiChroprep RP-18 (25–40-μm particle size, 100-Å pore size) from Merck, packed in powder form; (2) LiChrosorb RP-18 (7-μm particle size, 100-Å pore size) from Merck, packed by using the method of Manius & Tscherne (1981); (3) Aquapore RP-300 (10-μm particle size, 300-Å pore size) purchased from Brownlee Labs.

HPLC was carried out at room temperature (ca. 22 °C); effluent was collected by using an LKB Ultrarac Model 7000 fraction collector. 2-Propanol, acetonitrile, and ammonium acetate (HPLC quality) were purchased from J. T. Baker, and the water was quartz double distilled. For reduction of losses, the column effluent from a particular separation was diluted and loaded directly (through pump A) for the subsequent separation. Only following the final purification step was lyophilization used for sample recovery. Yields were determined by several methods: (a) by measuring peak areas manually or by automatic integration (Hewlett Packard Model 3309 A integrator) and comparing them with known amounts of purified proteins; (b) by amino acid analysis; and/or (c) by scintillation counting of ^{14}C-labeled parvalbumin which had been added prior to chromatography.

Protein Concentrations and Amino Acid Analysis. Protein concentrations of unpurified samples were measured by using the microbiuret method (Itzhaki & Gill, 1964). Amino acid analyses were carried out on a Durrum D-500 analyzer following hydrolysis at 110 °C for 22 h in 6 N HCl under vacuum.

Sodium Dodecyl Sulfate–Polyacrylamide Gels. Two-dimensional gel electrophoresis (O'Farrell, 1975) was used to minitor purification. Prior to sample application, the proteins were labeled by reductive methylation (Jentoft & Dearborn, 1979) by using [^{14}C]formaldehyde (specific activity 52 Ci/mol, New England Nuclear) and $NaCNBH_3$.

The isoelectric focusing gel contained a mixture of 20% Ampholine, pH 2.5–4, and 80% Ampholine, pH 3.5–10.0 (obtained from LKB), which resulted in a linear gradient from pH 3.8 to 6.1. Following electrophoretic development of the second dimension, the gels were fixed in acetic acid for 1 h and further processed for fluorography as described by Bonner & Laskey (1974) and Laskey & Mills (1975).

Ca^{2+} Analysis. For all experiments, only polypropylene pipets and containers which had been previously soaked in 0.1 M HCl and rinsed with quartz-distilled water (containing less than 10^{-7} M Ca^{2+}) were used. Ca^{2+} measurements were carried out following dialysis of the protein solution against quartz-distilled H_2O for 48 h at 4 °C in Spectrapor 3 membranes (Spectrum Medical Industries). A Perkin-Elmer atomic absorption spectrometer (Model 303) was used, and the standards were prepared from "spec pure" Ca^{2+} salts (Merck).

Immunological Methods. Antibodies against purified muscle parvalbumin were obtained by injecting rabbits intradermally with an emulsion of 0.5 mg/mL 0.9% NaCl and 1 mL of Freund's complete adjuvant. A second immunization (0.5 mg) and a third (1 mg), both in 1 mL of Freund's incomplete adjuvant, were made at 4-week intervals. The final subcutaneous boost (1 mg of protein in 1 mL of Freund's incomplete adjuvant) was given after an additional 2-week period, and blood was taken 2 weeks later. The serum was tested for its immunological specificity by double-immunodiffusion tests, the immunoreplica technique, immunoelectrophoresis, and immunoprecipitation as described by Celio & Heizmann (1981). These monospecific antibodies were used to compare muscle and brain parvalbumin in double-immunodiffusion tests. Since the mobility of the antigen is much higher than the one of the antibody, Ouchterlony plates had to be preincubated with antiserum for 4 h at room temperature prior to the application of the antigen.

Peptide Mapping. The HPLC instrument has been described above. An RP-18 column (10 μm, 100 Å) (Brownlee Labs) was used. Digestion was carried out for 7 h by using 4% (w/w) TPCK-trypsin at room temperature. The reaction medium (10 μL) contained 50 mM NH_4HCO_3 and 1 mM EGTA.

Results

In a previous study, we have reported the chromatographic behavior of various Ca^{2+}-binding proteins during reverse-phase HPLC using five different buffer systems (Berchtold et al., 1983). The suitability of this mode of chromatography was illustrated both by the high recoveries and by the ability to isolate the parvalbumins (isoproteins II and III) of pike muscle. These observations, as well as those reported in a recent study where the HPLC separation of various peptides/proteins was compared by using two different column types (Wilson et al., 1982a), indicated that HPLC might be a convenient method for the isolation of rat brain parvalbumin.

Isolation of Neuronal Parvalbumin. (*a*) *Extraction from Brain Tissue.* Homogenization of 20 rat brains (37.5 g) was performed at 4 °C in a Virtis blender (4 × 15 s, full speed) in 40 mL of 4 mM EDTA, pH 7.5, containing protease inhibitors (see Materials and Methods). After centrifugation (50000*g* for 30 min; each following centrifugation was identical), the pellet was reextracted by sonication (Branson Ultrasonic, 2 × 30 s at 100 W) in 40 mL of 50 mM Tris-HCl and 4 mM EDTA, pH 7.5, and centrifuged. Both supernatants (80 mL) were combined, $CaCl_2$ and 2-mercaptoethanol were added to 0.1 and 1 mM, respectively, and the pH was adjusted to 7.5. ^{14}C-Methylated muscle parvalbumin was added only for the measurement of protein recoveries of the subsequent purification steps (Table I) and for monitoring HPLC elution profiles (Figure 1). The solution was subsequently heated for 30 min at 85 °C and centrifuged. After being rapidly cooled (on ice) to 25 °C, an ammonium sulfate solution (662 g/L, corresponding to 90% saturation at 25 °C) was added to a final saturation of 45%. The suspension was centrifuged and the pellet washed with 50 mM Tris-HCl, pH 7.5, in 45% saturated ammonium sulfate. The supernatants were combined and either directly used for HPLC or stored at −20 °C.

(*b*) *Purification by HPLC.* A 160-mL sample of the final supernatant was directly loaded on a LiChroprep RP-18 column and eluted as shown in Figure 1a. Following extensive washing with 5% buffer B, we eluted parvalbumin by increasing the buffer B concentration isocratically to 40%; further elution with 100% buffer B failed to elute any material which either absorbed at 220 nm or contained radioactivity. Fractions were pooled (Figure 1a, see bar), diluted (1:5 with buffer A), and loaded on a second RP-18 column (Figure 1b) equilibrated in buffer A. After the column was washed, a

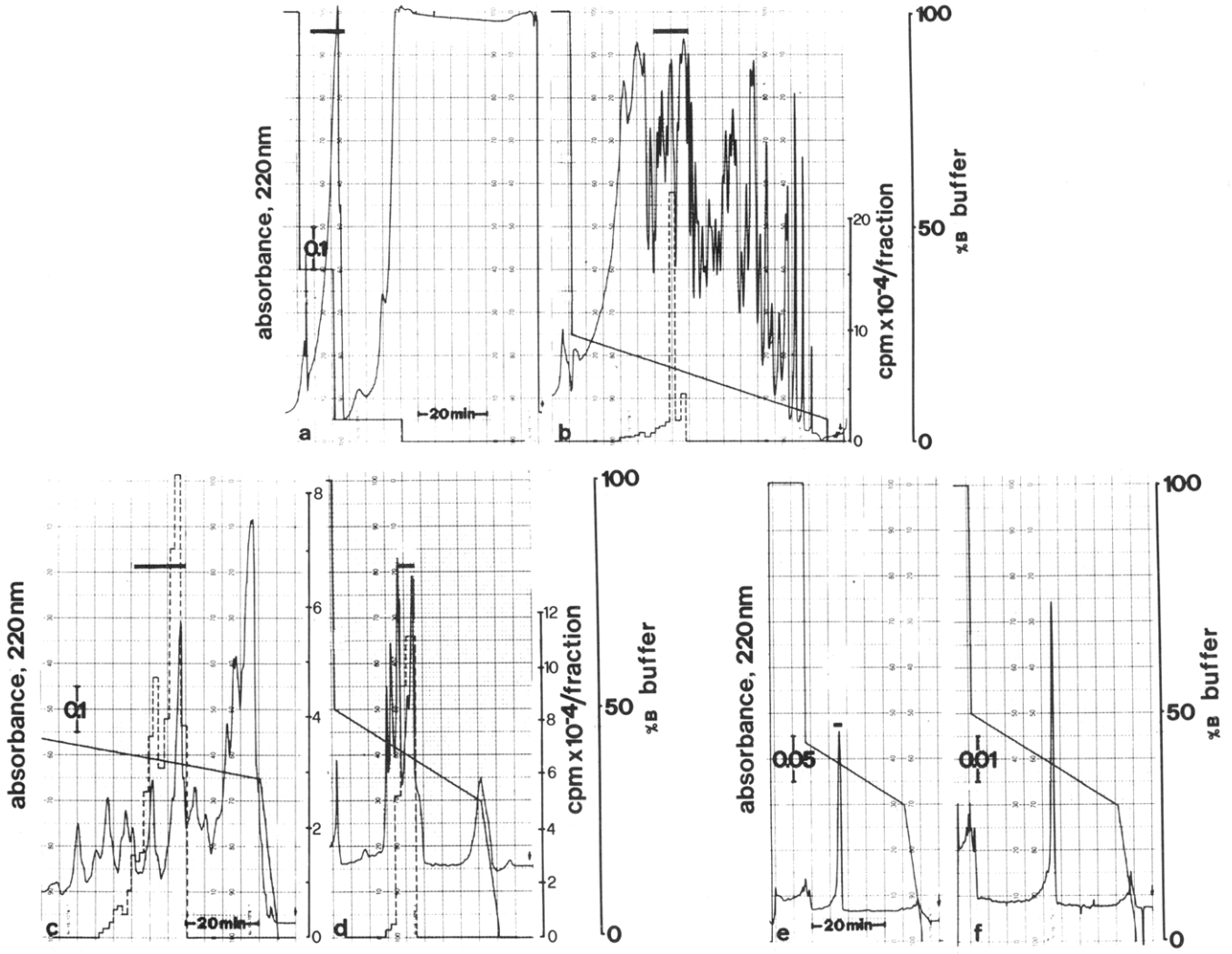

FIGURE 1: HPLC of final supernatant (160 mL) after heat treatment and ammonium sulfate precipitation. ^{14}C-Methylated muscle parvalbumin (70 μg × 455 000 cpm) was added as an internal marker prior to the heat treatment. Except for step 3 (b) where the pump pressure showed a maximum of 2000 psi, all chromatography runs were carried out under a back-pressure of approximately 700 psi. Arrows indicate sample injections. (a) Chromatography on a LiChroprep RP-18 column (9 × 500 mm) at a flow rate of 5 mL/min. Buffers were (A) 50 mM Tris-HCl, pH 7.5, and 0.1 mM $CaCl_2$ and (B) buffer A in 90% 2-propanol. Fractions were pooled (50 mL, see bar) and diluted prior to subsequent chromatography. (b) Isolation on a column of LiChrosorb RP-18 (4.6 × 250 mm) with the same buffer as in Figure 1a. Following sample application at 2 mL/min, the proteins were eluted at 0.75 mL/min with a linear gradient from 5 to 25% buffer B over 75 min. Aliquots were removed for cpm measurements and the fractions pooled accordingly (see bar). (c) Following dilution, the material was applied onto an Aquapore RP-300 column (4.6 × 250 mm) and elution carried out at a flow rate of 1 mL/min by using the same A buffer system as in Figure 1a with the B buffer containing 60% acetonitrile instead of 2-propanol. (d) Final purification of the pooled and diluted material was carried out as in (c) except that a buffer combination consisting of (A) 20 mM ammonium acetate and 0.1 mM $CaCl_2$ and (B) buffer A in 60% acetonitrile was employed. (e) and (f) illustrate analytical HPLC of the isolated material (67 μg) from brain and ^{14}C-labeled muscle parvalbumin (18 μg), respectively; chromatographic conditions identical with those in (d).

linear gradient from 5 to 25% buffer B was used to obtain an enriched parvalbumin fraction. The pooled fraction from Figure 1b was diluted to a final concentration of 4.5% 2-propanol and separated on an Aquapore RP-300 column (Figure 1c). Parvalbumin, eluting at 38.5–41.0% buffer B, was collected and in the final purification step chromatographed in a volatile buffer system (Figure 1d). Rechromatography (Figure 1e) indicated protein homogeneity and an elution pattern identical with that of ^{14}C-labeled muscle parvalbumin (Figure 1f). The shoulders in the elution profiles of added ^{14}C-labeled muscle parvalbumin (Figure 1b,c), and observed to disappear during further purification (Figure 1d,e), may be due to interactions of parvalbumin with other components present in the chromatographed material.

Table I summarizes the above HPLC-based isolation procedure. From 37.5 g of brain tissue, 246 μg of parvalbumin was obtained in an overall yield of 74%. Preparations starting with 2 or 8 g of brain tissue (results not shown) gave reduced average overall yields of 48.1 and 58.9%, respectively, with maximum deviations of 5% in three experiments. Increasing yields when higher loads were applied have also been noted for myoglobin, trypsin inhibitor, and carbonic anhydrase under similar HPLC conditions (Wilson et al., 1982a). For comparative purposes, muscle parvalbumin was isolated under identical conditions with a yield of 560–670 mg/kg. On the basis of these recoveries, it has been estimated that the tissue concentrations of parvalbumin are 9–10 and 750–900 mg/kg in brain and muscle, respectively.

The loss of protein was most noticeable during chromatography on the LiChrosorb column and the final stage of purification on the 300-Å pore size reverse-phase support. The application of variable sample volumes (up to 250 mL) and high salt concentrations [up to 45% saturated $(NH_4)_2SO_4$] had no adverse effects on either recovery or elution positions of the proteins. The columns used showed no significant loss of separation efficiency due to the loading with heat-treated

Table I: Purification of Neuronal Parvalbumin[a]

purification step	volume (mL)	[protein] (mg)	x-fold purification	radioactivity (cpm)	recovery yield (%)
EDTA extract	130	6600	1		
(1) supernatant, EDTA extraction	80	1408	4.7	454 740	100
(2) load, LiChroprep column	160	100.8	65.5	445 710	98
(3) load, LiChrosorb column	250	38.5	171.4	440 000	96.7
(4) load, Aquapore RP-300 column, Tris-HCl	37.5	9	733.3	388 930	85.5
(5) load, Aquapore RP-300 column, NH_4Ac	50	1.2	5500	371 320	81.7
(6) purified parvalbumin	6	0.25	26400	336 780	74.1

[a] Starting material was 37.5 g of rat brain tissue (20 animals) and 70 μg of ^{14}C-methylated muscle parvalbumin, which was added as an internal standard (454 740 cpm), thus allowing the determination of protein recoveries.

extracts even after more than 100 runs. However, we observed a slight shift of the elution pattern toward shorter retention times after prolonged column use, especially for step 3 (Table I).

For estimation of the capacity of the reverse-phase supports for steps 2 and 3 (Table I), the following experiments were carried out: rat brain extract (supernatant after heat treatment) mixed with ^{14}C-labeled muscle parvalbumin (20 000 cpm, 3 μg) was loaded on 1 g of Lichroprep RP-18, packed in a Pasteur pipet until radioactive material started to elute (indicating that the capacity was exceeded). A similar experiment was done with the collected material from the first column to find out the load limit for the C-18, 7-μm pore size, powder. The capacities were found to be 7.2 and 21.3 mg of loaded proteins per g of Lichroprep, 25–40-μm pore size, and Lichrosorb, 7-μm pore size, support, respectively. This results in load maxima of approximately 230 and 90 mg of heat-stable brain proteins for steps 2 and 3 (Table I).

As illustrated in Figure 2, the purification of neuronal parvalbumin was followed by two-dimensional gel electrophoresis. Total EDTA extract could be shown to contain minimally 100 distinct protein spots with brain parvalbumin being clearly recognizable by its typical pI of 4.9 and M_r of 12 000 (Figure 2a, arrow). After heat and ammonium sulfate treatment and two consecutive HPLC purification steps (Table I, steps 1–4), more than 20 low molecular weight proteins were still visible (Figure 2b). The final purification step (Table I, step 6) yielded homogeneous neuronal parvalbumin as judged by two-dimensional gels (Figure 2c) and HPLC (Figure 1e). Brain and muscle parvalbumin migrated identically (Figure 2d) with a molecular weight of 12 000 determined by using chicken and rabbit parvalbumin (both M_r 12 000) as well as protein standards with molecular weights ranging from 14 300 to 100 000.

Characterization and Comparison of Neuronal and Muscle Parvalbumin. Table II gives the amino acid analyses for brain and muscle parvalbumins, which both show low contents of proline (0), methionine (2–3), and histidine (2) and high phenylalanine (8–9), glutamic acid (9–10), and aspartic acid (14–15) values. The two analyses are very similar. The UV spectra (not given) of neuronal and muscle parvalbumin were

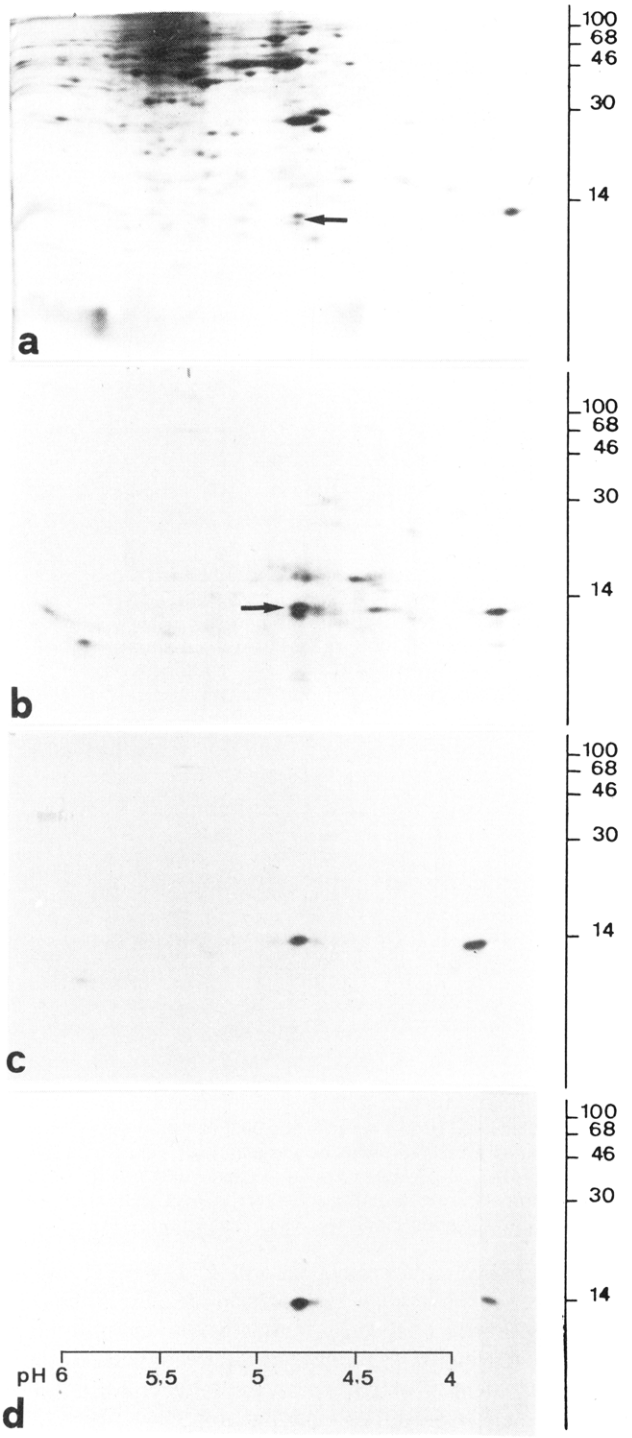

FIGURE 2: Two-dimensional polyacrylamide (15%) gel electrophoresis. Prior to being loaded, protein samples were ^{14}C labeled by reductive methylation. (a) Rat brain extract after centrifugation (see Results and Table I). Arrow indicates position of parvalbumin. (b) Parvalbumin-containing fraction (30 μg) eluted from the second RP-18 column (Table I, step 4, and Figure 1b). (c) Purified brain parvalbumin (0.1 μg; see Table I, step 6, and Figure 1e). (d) Mixture of brain and muscle parvalbumins, (0.1 μg of each). ^{14}C-Labeled proteins are identified by pI (scale on bottom) and M_r (values in kilodaltons) and were visualized by fluorography (1-day exposure). The single spot to the right side of each gel represents parvalbumin from rat muscle, run only in the second dimension.

identical and showed the typical phenylalanine absorbance, i.e., lack of both tyrosyl and tryptophanyl residues.

The Ca^{2+} contents of brain and muscle parvalbumins, (three independent measurements) determined by atomic absorption averaged 1.81 ± 0.2 and 1.92 ± 0.2 mol of Ca^{2+}/mol, re-

Table II: Amino Acid Compositions of Rat Brain and Muscle Parvalbumins

amino acid	brain parvalbumin[a]	muscle parvalbumin[a]
lysine	15.5	15.5
histidine	2.2	1.8
arginine	1.0	0.5
aspartic acid	14.6	14.4
threonine[b]	5.2	5.5
serine[c]	10.6	11.0
glutamic acid	9.5	10.0
proline	0	0
glycine	9.4	10.5
alanine	11.4	11.1
valine	5.2	5.1
methionine	2.4	2.1
isoleucine	6.0	5.8
leucine	9.6	9.8
tyrosine[d]	0	0
phenylalanine	8.5	8.1
tryptophan[d]	0	0
cysteine[e]	0	0

[a] 22-h hydrolysis; average of two and three analyses from separate preparations of the brain and muscle proteins, respectively; an M_r of 12 000 was used for calculations. [b] Assuming loss of 7% during hydrolysis. [c] Assuming loss of 9% during hydrolysis. [d] Obtained from spectroscopic data. [e] Determined after oxidation with performic acid (Hirs, 1967).

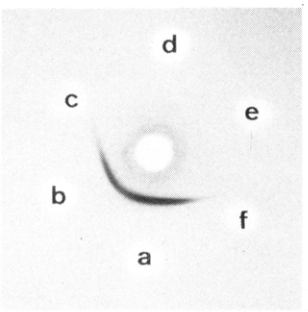

FIGURE 3: Double-immunodiffusion test of antimuscle parvalbumin serum (center well, 10 μL) against neuronal parvalbumin (a), muscle parvalbumin (b), S-100 protein (bovine brain) (c), calmodulin (bovine brain) (d), troponin C (rabbit muscle) (e), and combined Ca^{2+}-binding proteins from sandworm and crayfish (f). Outer wells contained 1 μg of proteins. Amide black was used for staining.

spectively. Brain and muscle parvalbumins were also tested by Ouchterlony double immunodiffusion (Figure 3) by using specific antiserum against rat muscle parvalbumin. A single precipitin line (with no spurs) was observed, demonstrating serological identity of both proteins. Structurally related proteins such as S-100, calmodulin, troponin C, and Ca^{2+}-binding proteins from sandworm and crayfish were unreactive. This agrees with Van Eldik & Watterson (1981), who demonstrated cross-reactivity of anti-calmodulin with calmodulin from other species but not with parvalbumin or troponin C. Peptide maps of muscle parvalbumin isolated by conventional methods and neuronal parvalbumin purified in preliminary experiments by using acid buffer systems (A, 0.1% H_3PO_4 and 10 mM $NaClO_4$; B, buffer A in 60% acetonitrile, pH 2.1) showed differences in the retention times of three peaks. Amino acid analysis of these peptides revealed that they all contain methionine (data not shown). These findings suggested that the chromatographic differences might be due to isolation artifacts arising from the use of the acid HPLC buffer system (see above) and possibly in oxidation of methionyl residues. In the isolation procedures for neuronal parvalbumin described here, only neutral buffer systems were therefore used. Complete identity in the peptide maps of muscle and neuronal parvalbumins was obtained (Figure 4). Identical protein hydrophobicities were demonstrated by the same retention times on a C-18 reverse-phase HPLC column (Figure 1e,f).

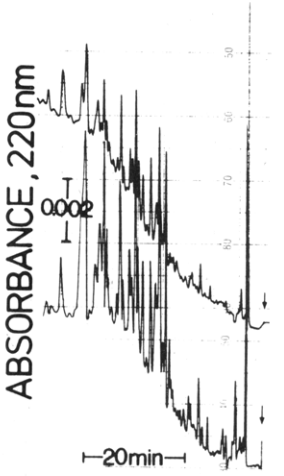

FIGURE 4: Tryptic peptide maps of neuronal (upper trace) and muscle (lower trace) parvalbumins. An RP-18 10-μm column and the following buffer systems were used for mapping: (A) 0.1% H_3PO_4–10 mM $NaClO_4$; (B) (A) in 60% acetonitrile. The buffer gradient was 0–45% (B) in 27 min and 45–60% (B) in 23 min. Peptide amounts were 0.45 (neuronal parvalbumin) and 0.8 nmol (muscle parvalbumin). The back-pressure was approximately 700 psi.

Discussion

By application of high-performance liquid chromatography on reverse-phase supports, it has been possible to isolate parvalbumin from rat brain tissue. A 26 400-fold purification was achieved by heat and ammonium sulfate precipitation and a subsequent series of four HPLC isolations under different conditions (buffers, gradients, columns). This resulted in a homogeneous preparation of neuronal parvalbumin. On the basis of protein recovery measurements, the content per gram wet weight has been estimated to approximately 100 times lower than that in muscle extracts from the same species.

Compared to the more traditional isolation procedures (gel and ion-exchange chromatography) used for the purification of parvalbumin (or other Ca^{2+}-binding proteins), HPLC offers several advantages:

(a) Protein recoveries are considerably higher (at least 2-fold, depending upon the amount of starting material), an essential point either when the parvalbumin(s) is (are) present in low concentrations (e.g., brain) or when only limiting amounts of starting material are available (e.g., human tissues).

(b) Total isolation of parvalbumin to homogeneity can be carried out within 1 day. This is 5–7 times faster than the more conventionally employed methods.

(c) Isolation can be performed by using organic solvents, at low salt concentrations and at physiological pH conditions which do not appear to alter the physical properties of the isolated product. This is demonstrated by the identity of the tryptic peptide maps of muscle parvalbumin, isolated by HPLC or by conventional methods (not shown). The activity of calmodulin as an activator of phosphodiesterase was found to be unchanged following HPLC under identical conditions (Berchtold et al., 1983). However, the use of low-pH buffer systems completely destroyed this activity. Neuronal parvalbumin isolated under such conditions exhibited an altered peptide map, differing in those peptides which contained methionyl residues.

(d) Time-consuming dialysis and/or lyophilization steps which potentially result in protein loss, i.e., physical loss due

to sticking to dialysis membranes/lyophilization flasks or through denaturation, could be circumvented by direct loading of the samples from the previous separation (after sufficient dilution to lower the concentration of the organic modifier) onto the following.

(e) The method is also applicable as a means of determining protein homogeneity. As little as 0.1 nmol (1.2 μg) of protein could be detected and separated from other Ca^{2+}-binding proteins (calmodulin, S-100 protein, or troponin C) which are often present in the same tissue extracts.

A direct comparison of the neuronal and muscle parvalbumins from the rat revealed identities in their molecular weights, isoelectric points, UV spectra, Ca^{2+} contents, and immunological properties as well as in their amino acid compositions and retention times on reverse-phase supports. A comparison of the tryptic peptide maps of both proteins carried out by HPLC also indicates total identity. By this method, very small hydrophobicity differences, for example, two PS-1 peptides both containing 27 amino acids and differing only at one position (leucine instead of valine), could be separated by using identical chromatography conditions (Wilson et al., 1981b).

From the sequence data for the calmodulins from bovine uterine muscle (Grand & Perry, 1978) and bovine brain (Watterson et al., 1980), only differences in the amidation states of aspartyl residues at positions 24 and 97 have been found. This suggests a similar tissue nonspecificity to that of parvalbumin. Several proteins earlier believed to be muscle specific, e.g., myosin, actin, and tropomyosin, were later also found in brain, however, clearly differing in their primary structures [for reviews, see Thompson (1976), Grand & Perry (1978), Gröschel-Stewart (1980), and Bray & Gilbert (1981)]. Although neuronal and muscle parvalbumins appear to be very similar by the above criteria, the latter is probably involved in the fast muscle relaxation (Pechère et al., 1977; Blum et al., 1977; Fischer et al., 1976; Gillis & Gerday, 1977; Haiech et al., 1979; Gillis, 1980; Celio & Heizmann, 1982), though this has been questioned recently (Robertson et al., 1981). Brain parvalbumin is thought to be involved in Ca^{2+}-dependent processes in a distinct subpopulation of neurons (Celio & Heizmann, 1981).

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