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ION-EXCHANGE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC PURIFICATION OF BOVINE CARDIAC AND RABBIT SKELETAL MUSCLE TROPONIN SUBUNITS

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SUMMARY

Bovine cardiac and rabbit skeletal troponin complexes were separated into their respective subunits employing high-performance liquid chromatographic (HPLC) techniques on CM-300 and Q-300 ion-exchangers. Bovine cardiac and rabbit skeletal subunits were separated on the strong anion-exchanger, Q-300, in 8 *M* urea, 50 m*M* Tris, 2 m*M* EGTA, 0.5 m*M* dithiothreitol, pH 7.5, employing a linear salt gradient and on the weak cation-exchanger, CM-300, in 8 *M* urea, 50 m*M* potassium dihydrogen phosphate, 2 m*M* EGTA, 0.5 m*M* dithiothreitol, pH 6.5, using a linear salt gradient. To obtain complete purification of all components of troponin both ion-exchangers were required. The initial separation of troponin was carried out on the strong anion-exchanger followed by weak cation-exchange chromatography of the troponin I collected from the strong anion-exchange column. The troponin T subunits obtained from Q-300 chromatography demonstrated heterogeneity (three components: T1, T2 and T3) while the troponin I collected from both sources on the Q-300 column were both resolved into major doublets (I1 and I2) when rechromatographed on the CM-300 column. The three troponin T fractions and two troponin I fractions isolated from ion-exchange HPLC were examined by sodium dodecyl sulfate—urea polyacrylamide gel electrophoresis and two-dimensional gel electrophoresis to confirm that the heterogeneity was due to differences in charge and not molecular weight. These results were in agreement with the charge differences observed from retention times on ion-exchange HPLC. When comparing the same troponin subunit from different muscle sources, considerable differences in the content of charged amino acid residues were also observed.

INTRODUCTION

Ebashi et al. [1] were the first to show that native tropomyosin consisted

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of two proteins, tropomyosin and troponin, both of which were required to confer Ca^{2+} sensitivity to purified actomyosin preparations. As well, these studies demonstrated that it was the troponin component which was the Ca^{2+} receptor of native tropomyosin. Hartshorne and Mueller [2] further separated troponin into two components, which were called troponin A and troponin B; both of these, in addition to tropomyosin, were required to confer Ca^{2+} sensitivity to synthetic actomyosin. Similar results were also obtained by Schaub and Perry [3] using sulfoethyl (SE)—Sephadex chromatography in the presence of 6 *M* urea. Greaser and Gergely [4, 5] employed sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) and DEAE—Sephadex chromatography in the presence of 6 *M* urea to achieve the separation of troponin into three subunits which they called troponin T (TnT), troponin I (TnI) and troponin C (TnC). All three subunits, in addition to tropomyosin, were required to reconstitute native tropomyosin activity. Potter [6] has published two detailed descriptions of the purification of both cardiac and skeletal troponin complexes using Cibacron Blue—Sephacryl chromatography. This procedure, followed by various combinations of chromatography on DEAE—Sephadex and CM—Sephadex in the presence of 6 *M* urea and 1 m*M* EDTA can also be used to prepare pure troponin subunits. Our laboratory has used modifications [7] of the method of Staprans et al. [8] for the purification of native rabbit skeletal troponin complex and its subunits, while the purification of bovine cardiac troponin complex and its subunits has been accomplished using methods worked out by Burtnick and co-workers [9—11]. All these methods require open-column chromatography on ion-exchange resins such as CM—Sephadex and DEAE—Sephadex and size-exclusion chromatography. The methods are successful in providing pure forms of the native troponin complex as well as the subunits from both the bovine cardiac and rabbit skeletal systems. There is a disadvantage, however, in that these methods of purification are time-consuming and result in the dispersion of the desired product(s) in large volumes of column effluent. Recently, Gusev et al. [12] have used DEAE—cellulose chromatography in the presence of 8 *M* urea to separate bovine cardiac TnT into multiple peaks. The heterogeneity observed was shown to result from the presence of two forms of TnT, differing in their molecular weight (M_r) values, amino acid content, degree of phosphorylation and aggregation. We have shown by use of high-performance liquid chromatographic (HPLC) techniques that both bovine cardiac and rabbit skeletal troponin complexes can be separated into their respective components employing CM-300 and Q-300 resins in 8 *M* urea and that this method of purification allows for the rapid separation of the troponin complexes not only into their respective subunits but resolution of the subunits TnI and TnT into multiple peaks.

EXPERIMENTAL

Reagents

Deionized water used in these experiments was further purified on a Milli-Q system supplied by Millipore. The urea used was certified ACS grade urea supplied by Fisher Scientific as were the potassium chloride and potassium

dihydrogen phosphate. Ethyleneglycol-bis(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA) was purchased from Sigma while dithiothreitol (DTT) was obtained from the Bethesda Research Labs. Millipore filters were purchased from Millipore. Analytical-grade mixed-bed resin AG 501-X8 (20–50 mesh, fully regenerated) was provided by Bio-Rad Labs. as was Bio-Rad Chelex-100 (50–100 mesh, Na^+). Ampholine 3.5—10 was purchased from LKB Bromma (dry content 40%, w/v) for the preparation of non-equilibrium pH gradient electrophoresis gels. Nonidet P40, used in these gels and in the sample dissolving solution, was purchased from Shell Chemicals. The Tris buffer and urea used to prepare gels and buffer solutions for gel analyses were ultra-pure-grade reagents purchased from Canadian Scientific Products. Acrylamide (> 99.9%) was a product from Bio-Rad Labs. and the N,N'-methylenebis-acrylamide (electrophoresis grade) was supplied by Bethesda Research Labs. Gels were stained with Coomassie Brilliant Blue from Sigma (Lot No. 112F-0102).

Buffer preparation for HPLC

Buffers were prepared in 4-l volumes to avoid variations in buffer conditions during individual experiments. It was necessary to remove UV-absorbing contaminants from reagent-grade urea by treating stock solutions of 8 *M* urea with AG 501-X8 mixed-bed resin (25 g/l) for 15 min at room temperature. As reported by Karkas et al. [13] stock solutions of 1 *M* potassium dihydrogen phosphate used to prepare 50 m*M* buffers also contained UV-absorbing impurities which were removed by treatment of the stock solution with Chelex-100 (10 g/l) for 8 h at 4°C. In order to assure that the reducing power of the buffers was maintained during the purification of large amounts of protein, 1-l portions of the stock buffers were made 0.5 m*M* in DTT. This buffer solution was then discarded at the end of the day and fresh buffer was then prepared. All buffer solutions were filtered through Millipore GS type 0.22-μm filters in a 90-mm stainless-steel filter cassette.

Two-dimensional gel electrophoresis

Non-equilibrium pH gradient electrophoresis was conducted according to the method of O'Farrell et al. [14]. The gels for the first dimension (13 cm × 1.5 mm) were made as follows: 0.7 ml acrylamide (acrylamide—bisacrylamide, 28.4:1.6), 2.75 g urea, 2.0 ml water, 0.25 ml Ampholine (LKB, pH 3.5—10, 40%, w/v), 0.12 ml NP-40, 20 μl 10% ammonium persulfate and 10 μl of N,N,N',N'-tetramethylethylenediamine (TEMED) were made up to a final volume of 5.1 ml. The gels were allowed to polymerize for 1 h before use. Samples were dissolved immediately before application in the following solution: 10 g/l β-mercaptoethanol (BME), 20 g/l NP-40, 1 m*M* EGTA and 8 *M* urea. This solution is a modification of the sample-dissolving solution used by Giometti et al. [15]. Samples consisted of 1 mg (by weight) of protein dissolved in 1.0 ml of the dissolving solution. Aliquots (10 μl) of these samples were applied directly to the acidic end (top) of the gels and the electrophoresis was carried out towards the cathode. The electrode vessels were filled as follows: the upper reservoir was filled with 0.01 *M* phosphoric acid and the bottom reservoir with 0.02 *M* sodium hydroxide. The gels were run at constant

voltage (500 V) for 5 h for a total of 2500 V-h. At this time they were carefully extruded under pressure and equilibrated for 20 min in a 10-ml solution containing 50 ml glycerol, 25 ml BME, 250 ml of a buffer composed of 0.2 *M* Tris · HCl, 0.2% SDS (w/v), pH 6.8, 175 ml water and 0.2% bromophenol blue. After equilibration of the gels, they were layered on Laemmli gels for the second-dimension separation. Laemmli urea gel electrophoresis was performed by the method of Laemmli [16] except that the separating gel was formed from 15% polyacrylamide, 0.1% SDS and 6 *M* urea in 0.465 *M* Tris · HCl buffer, pH 8.8. The stacking gel was formed from a 4% polyacrylamide, 0.1% SDS and 6 *M* urea in 0.13 *M* Tris · HCl buffer, pH 6.8. These gels were electrophoresed for 20 h at a constant current (25 mA). The gels were then placed in destain solution (methanol—acetic acid—water (10:10:80) for 1 h and then stained for 1 h in methanol—acetic acid—Coomassie Brilliant Blue R-250 (Sigma)—water (50:10:0.25:40, w/v). The gels were then destained in methanol—acetic acid—water (40:10:50) for 2 h at which point they were placed in methanol—acetic acid—water (10:10:80) until the background was clear.

pH Gradient measurements (first dimension)

pH Gradients in the non-equilibrium pH gradient electrophoresis gels were measured in the following manner. In each experiment a control tube gel was included with the sample gels. At the end of the run this gel was extruded from the electrophoresis tube and was cut into 1-cm lengths. These gel pieces were immediately placed in 1 ml of deionized water and were allowed to equilibrate in this solution for 1 h. At the end of this time the pH of each solution was measured using a Radiometer (Copenhagen) Model PHM62 standard pH meter. All first-dimension gels for this analysis were run over the pH range 3.5—10.

Sample preparation for HPLC

Samples prepared for analysis on SynChropak CM-300 resin were dissolved in 8 *M* urea, 50 m*M* potassium dihydrogen phosphate, 2 m*M* EGTA, 0.5 m*M* DTT and the pH of the sample was adjusted to pH 6.5 by the addition of 1-μl aliquots of either 5 *M* hydrochloric acid or 5 *M* potassium hydroxide. The samples to be chromatographed on SynChropak Q-300 resin were dissolved in 8 *M* urea, 50 m*M* Tris, 2 m*M* EGTA, 0.5 m*M* DTT and the pH was then adjusted to pH 7.5 as above. Samples dissolved in these buffers were centrifuged at 12 000 *g* for 5 min before injection on a column. The concentration of these samples was estimated by spectrophotometry at the appropriate wavelength using the values quoted in Table I.

HPLC and equipment

Columns were equilibrated for 15 min in the starting buffer before each run. Flow-rates were maintained at 1 ml/min (analytical chromatography) and 2 ml/min (preparative chromatography). All chromatograms were obtained at ambient temperature (ca. 22°C) and protein fractions were collected manually.

Programmed analytical chromatographic runs were performed on a Varian Vista Series 5000 liquid chromatograph interfaced with a Varian CDS401 data system. The chromatograph was coupled to a Kratos SF769Z variable-

TABLE I

PROTEIN EXTINCTION COEFFICIENTS AND MOLECULAR WEIGHTS

Protein	Extinction coefficient (1 mg/ml)	Wave-length (nm)	Molecular weight	Authors	Reference No.
Rabbit skeletal troponin	0.47	280	70 000*	Lovell and Winzor (1977)	17
Rabbit skeletal troponin C	0.19	280	18 000	Margossian and Cohen (1973)	18
Rabbit skeletal troponin I	0.59	280	21 000	Margossian and Cohen (1973)	18
Rabbit skeletal troponin T	0.50	280	30 500	Eisenberg and Kielley (1974)	19
Bovine cardiac troponin	0.43	280	77 500*	Byers et al. (1979)	20
Bovine cardiac troponin C	0.23	276	18 416	Byers and Kay (1982) Byers and Kay (1983)	21 22
Bovine cardiac troponin I	0.52	276	23 000	Byers and Kay (1982) Byers and Kay (1983)	21 22
Bovine cardiac troponin T	0.44	276	36 000	Byers and Kay (1982) Byers and Kay (1983)	21 22

*Sum of the three subunits.

wavelength UV spectrometer operated at 220 nm. Analytical samples were chromatographed on a SynChropak CM-300 weak cation-exchange column (250 mm × 4.1 mm I.D.) or a SynChropak Q-300 strong anion-exchange column (250 mm × 4.1 mm I.D.) purchased from SynChrom (Linden, IN, U.S.A.). Sample injections were made from a Hamilton No. 702 25-μl syringe (Hamilton, Reno, NV, U.S.A.) into a 200-μl injection loop (Model No. 7125, Rheodyne, Berkeley, CA, U.S.A.).

Programmed preparative chromatographic runs were performed using the same equipment as that described for the analytical runs. Preparative samples were chromatographed on a SynChropak Q-300 strong anion-exchange column (250 × 10 mm I.D.) purchased from SynChrom. Sample injections were made from a Hamilton No. 1750 500-μl syringe into a 2.0-ml injection loop (Model No. 7125, Rheodyne).

The chromatograms from both the analytical and preparative runs were recorded on a Molytek dual-pen recorder Model No. 3322 purchased from Technical Marketing Assoc. (Calgary, Canada).

Purification of bovine cardiac and rabbit skeletal troponin and their subunits by conventional techniques

Native rabbit skeletal troponin was purified using the method of Staprans et al. [8] with modifications according to Chong and Hodges [7]. Subunits TnC, TnI and TnT were prepared from native troponin using the method of

Greaser and Gergely [5] as modified by Chong and Hodges [7]. Initial separation of the major protein fractions from crude troponin was obtained by DEAE—Sephadex A-25 chromatography in 6 *M* urea. TnI obtained in this way was 80—90% pure as judged by SDS-PAGE and was used without further purification. TnT from the DEAE—Sephadex A-25 purification was found to be 75—85% pure and was subjected to final purification on SE—Sephadex at pH 8.0 in 6 *M* urea to provide a homogeneous product. TnC obtained directly from DEAE—Sephadex chromatography was homogeneous as judged by SDS-PAGE.

Native bovine cardiac troponin was prepared according to the method of Tsukui and Ebashi [23] as modified by Burtnick [24] and reported by Byers et al. [20]. Isolation of the subunits was carried out as follows: TnC was isolated according to the procedure of Burtnick et al. [9] from crude troponin by chromatography on DEAE—Sephadex A-25 in 6 *M* urea and was obtained directly as a homogeneous peak as determined by SDS-PAGE. TnI isolated on DEAE—Sephadex A-25 as above was then rechromatographed on CM—Sephadex C-25 in 8 *M* urea and high-molecular-weight contaminants were then removed by chromatography on Biogel P-200 in 8 *M* urea [10]. TnT was isolated as crude material on DEAE—Sephadex A-25 and was rechromatographed on CM—Sephadex C-25 in 8 *M* urea. Final desalting was carried out on Biogel P-2 in 5% formic acid [11].

RESULTS AND DISCUSSION

HPLC results

Fig. 1 demonstrates the separation of native bovine cardiac (A) and rabbit skeletal troponin (B) complexes on a SynChropak CM-300 weak cation-exchange column. In both cases the TnC component was eluted in the void volume (peak No. 1, Fig. 1A and B) and was not retained on the column. Heterogeneity was observed in both the TnI and TnT subunits from both sources. Examination of the fractions from the bovine cardiac complex using Laemmli urea—SDS-PAGE [16] (data not shown) indicated that separation had occurred between the three subunits and that peaks 2, 3 and 4 were TnT and that peaks 5 and 6 were TnI. Gel analysis also demonstrated that of the TnT peaks isolated only peak 2 contained pure TnT while peaks 3 and 4 were contaminated with TnI. A similar examination of the fractions collected from the chromatography of rabbit skeletal complex (Fig. 1B) indentified peaks 2 and 3 as TnI while peaks 4 and 5 were found to belong to TnT. The rabbit skeletal fractions did not exhibit the cross-contamination observed in the bovine cardiac samples. As shown in Fig. 1, a higher salt concentration is required in the gradient for the elution of the last troponin subunit from bovine cardiac troponin as compared to the last component of rabbit skeletal troponin. Interestingly, the relative positions of TnI and TnT in both samples are different. TnI elutes first, followed by TnT in rabbit skeletal troponin while TnI elutes after TnT in bovine cardiac troponin. The TnI components from bovine cardiac troponin were eluted at a potassium chloride concentration of approximately 0.20 *M* compared with approximately 0.15 *M* for TnI components from rabbit skeletal troponin. These results would indicate that

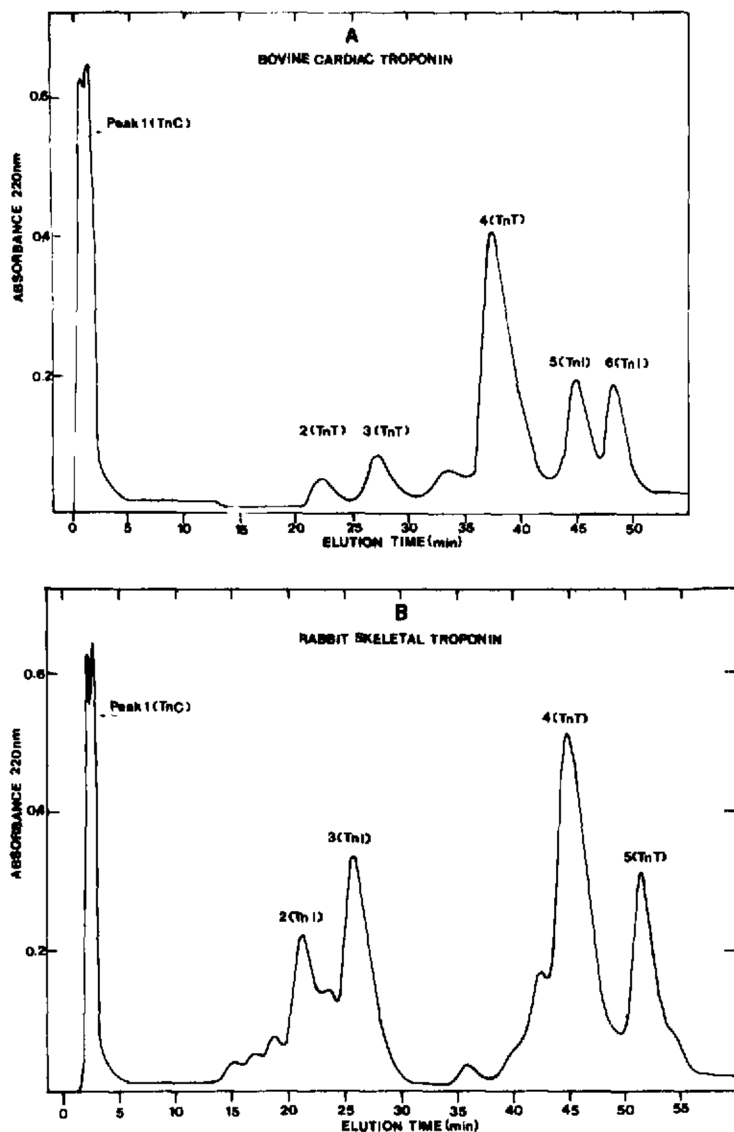

Fig. 1. Separation of bovine cardiac troponin (A) and rabbit skeletal troponin (B) on an analytical SynChropak CM-300 weak cation-exchange column. Column: 250 × 4.1 mm I.D. Conditions: A—B gradient, the basic buffer consisted of 8 *M* urea, 2 m*M* EGTA, 0.5 m*M* DTT, 50 m*M* potassium dihydrogen phosphate buffer, pH 6.5; buffer A was the basic buffer with no potassium chloride while buffer B consisted of the basic buffer containing 1 *M* potassium chloride. A linear potassium chloride gradient, with the potassium chloride increasing at a rate of 2 m*M*/min and a flow-rate of 1 ml/min was applied with the following compositions at the times indicated. Bovine cardiac and rabbit skeletal troponin gradient: time 0 min, 90% A—10% B; time 60 min, 78% A—22% B. Sample: 3 mg troponin per 100 μl of starting buffer. Chart speed: 12 in./h. Absorbance was measured at 220 nm, 10-mm cell.

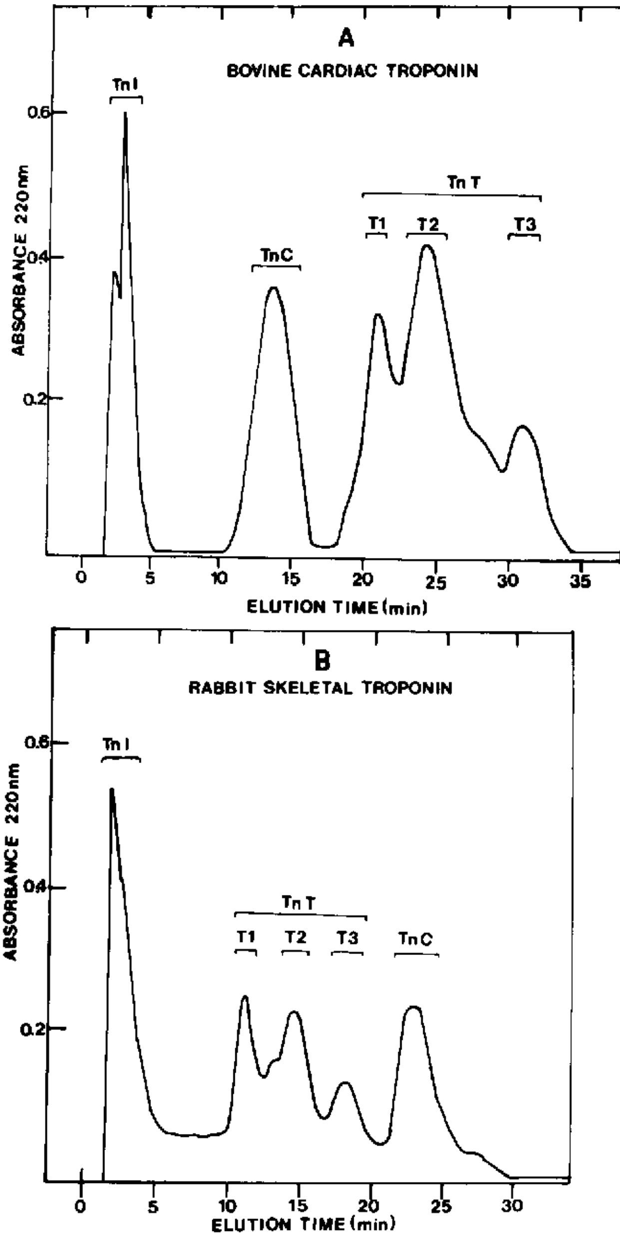

Fig. 2. Separation of bovine cardiac troponin (A) and rabbit skeletal troponin (B) on an analytical SynChropak Q-300 strong anion-exchange column. Column: 250 × 4.1 mm I.D. Conditions: A—B gradient, the basic buffer consisted of 8 *M* urea, 50 m*M* Tris, 2 m*M* EGTA, 0.5 m*M* DTT, pH 7.5; buffer A was the basic buffer with no potassium chloride, while buffer B consisted of the basic buffer containing 1 *M* potassium chloride. A linear potassium chloride gradient with the potassium chloride increasing at a rate of 5 m*M*/min and a flow-rate of 1 ml/min was applied with the following compositions at the times indicated. Bovine cardiac troponin gradient: time 0 min, 80% A—20% B, time 40 min, 60% A—40% B; Rabbit skeletal troponin gradient: time 0 min, 90% A—10% B, time 40 min, 70% A—30% B. Sample: 25 mg troponin per 200 μl of starting buffer. Chart speed, 12 in./h. Absorbance was measured at 220 nm, 10-mm cells.

the bovine cardiac TnI is more basic than the skeletal protein (see Fig. 5). In contrast, TnT components from bovine cardiac troponin were eluted at a potassium chloride concentration of approximately 0.17 *M* compared with approximately 0.20 *M* for the TnT components from rabbit skeletal troponin. Thus bovine cardiac TnT is less basic than the skeletal protein.

Owing to the cross-contamination of TnI in some of the TnT components, we examined the separation of the two troponin complexes on the strong anion-exchange column SynChropak Q-300. Fig. 2 represents the chromatographic separation of bovine cardiac (A) and rabbit skeletal (B) troponin, respectively. The TnT components T1, T2 and T3 from bovine cardiac troponin were eluted at considerably higher potassium chloride concentrations (approximately 0.33 *M*) than those from rabbit skeletal troponin (approximately 0.18 *M*). These results would indicate that the bovine cardiac TnT is much more acidic than the skeletal protein. This is consistent with the findings of Burtnick et al. [11] who have shown that cardiac TnT appears to be richer in acidic amino acid residues, which would explain the stronger binding to the anion-exchanger at pH 7.5. Interestingly, the relative positions of elution of TnT and TnC in both samples are different. TnT elutes first, followed by TnC in rabbit skeletal troponin while TnT elutes after TnC in the bovine cardiac troponin. Chromatography of purified samples of TnC from both sources under identical conditions showed that both samples have similar retention times. The primary sequence and p*I* values of the two TnC components are fairly similar (p*I* 4.1—4.3 [25]). Once again employing gel analysis as before we were able to examine the nature and purity of each of the peaks associated with these chromatograms. All fractions were homogeneous and no cross-contamination was observed between various subunits. As shown in Fig. 3, the heterogeneity observed in the TnT subunit is not due to TnI—TnT complex formation. Presumably these forms of TnT were separated during our

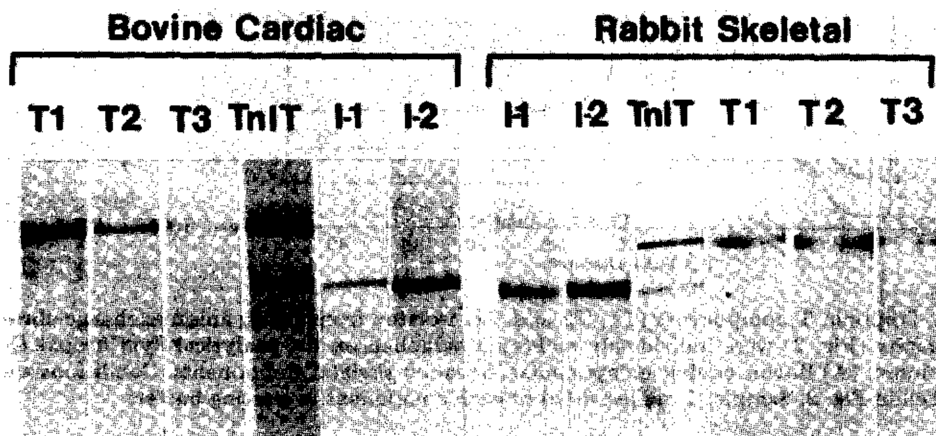

Fig. 3. Urea SDS-PAGE of bovine cardiac and rabbit skeletal HPLC fractions of troponin T and troponin I. The gel electrophoresis was performed according to the method of Laemmli [16]. The TnT fractions T1, T2 and T3 arise from strong anion-exchange HPLC on a Q-300 column (Fig. 2). The TnI fractions I1 and I2 arise from final purification on weak cation-exchange HPLC on a CM-300 column (Fig. 5).

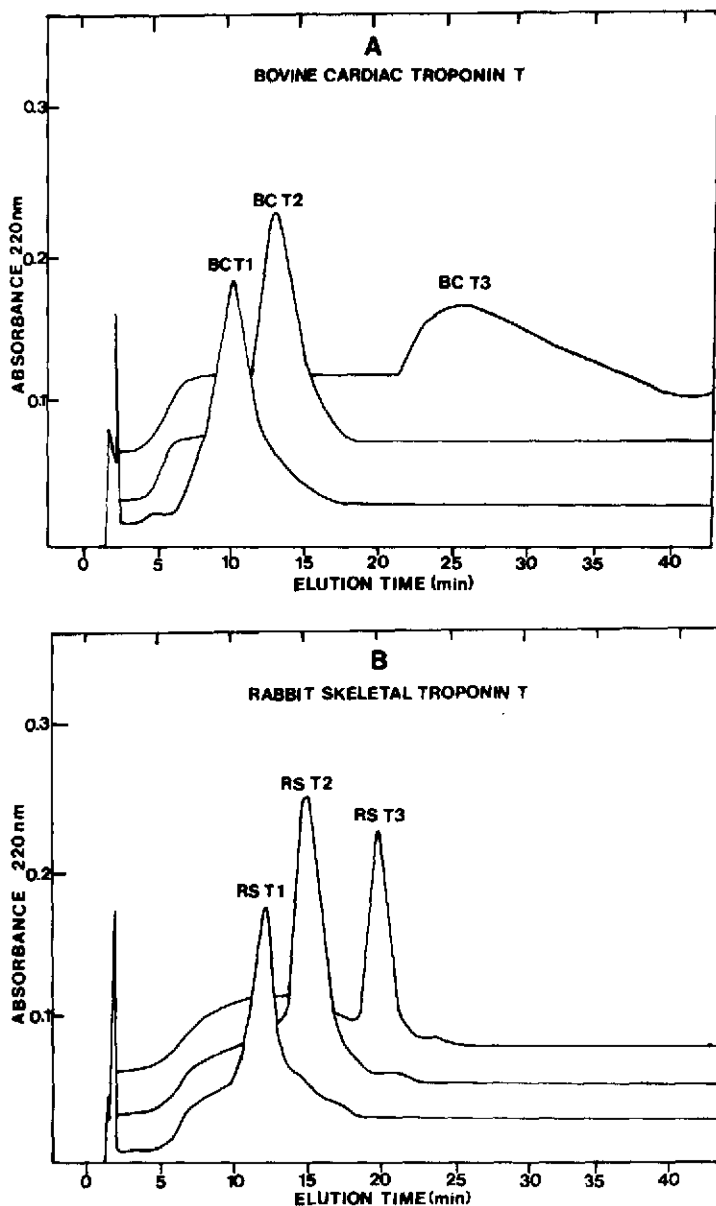

Fig. 4. Troponin T components (T1, T2 and T3) isolated from strong anion-exchange chromatography (Fig. 2) were individually rechromatographed on the analytical SynChropak Q-300 column. (A) Bovine cardiac components; (B) rabbit skeletal components. Conditions are described in Fig. 2. Sample: 1 mg per 50 μl of each component in starting buffer.

chromatographic runs on the basis of charge differences existing between subpopulations. Each of the TnT components was isolated and rechromatographed on the Q-300 support using the same conditions to demonstrate their uniqueness (Fig. 4). Although the conditions used were identical to those used during the isolation of each of the TnT fractions from both sources the peaks (T1, T2 and T3) isolated from the bovine cardiac complex exhibited a reduced

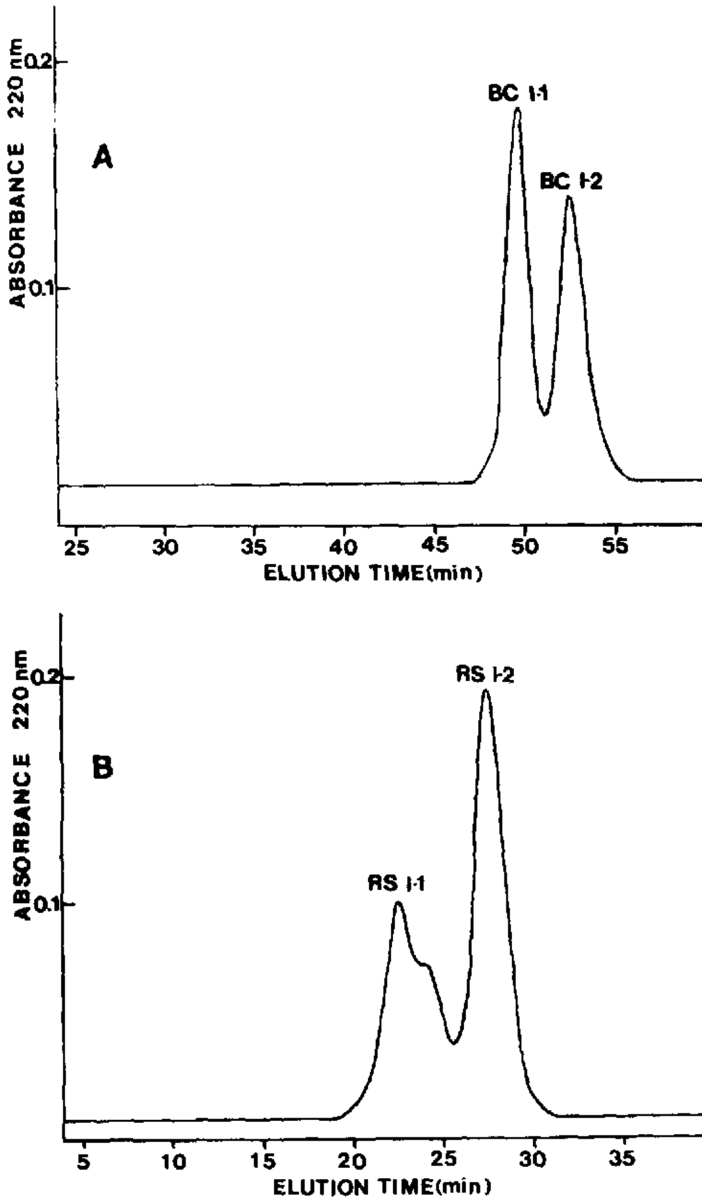

Fig. 5. Separation of bovine cardiac troponin I (A) and rabbit sekeletal troponin I (B) on an analytical SynChropak CM-300 weak cation-exchange column after isolation from strong anion-exchange chromatography (Fig. 2). Conditions are described in Fig. 1. Sample: 1 mg of TnI per 100 μl dissolved in starting buffer.

retention time when they were reinjected on this column (Fig. 4). The retention times of the TnT fractions from bovine cardiac troponin were uniformly reduced by approximately 5 min when they were examined in isolation from the rest of the complex. The rabbit skeletal fractions, however, exhibited retention times which were similar to those found during their isolation from

the complex. Both the bovine cardiac and rabbit skeletal TnC fractions isolated from the Q-300 column exhibited single peaks when they were reinjected on the Q-300 column.

On the Q-300 column, TnI from both sources was not retained and was eluted with the void volume (first peak, Fig. 2A and B) but this subunit was bound to the CM-300 column. For this reason TnI fractions collected from Q-300 chromatography were then purified on the CM-300 column (Analytical, 250 mm × 4.1 mm). As can be seen in Fig. 5A and B, bovine cardiac or rabbit skeletal TnI was resolved into two major components. Each of these components was isolated and reinjected under the same conditions on the CM-300 column to demonstrate their uniqueness. The purity of the TnI fractions was demonstrated using Laemmli [16] urea SDS-PAGE (Fig. 3). Heterogeneity in the TnI and TnT subunits from bovine cardiac and chicken-fast-skeletal muscle has been observed previously [12, 26—30].

Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis has been shown to be a powerful tool for the analysis of muscle complex components [15, 26, 27, 29]. In this method, introduced by O'Farrell et al. [14], non-equilibrium pH gradient electrophoresis is performed in the first dimension to separate components on the basis of charge. This is then followed by separation by molecular weight on SDS urea slab gels according to the method of Laemmli [16].

Using this method it has been demonstrated that TnT is composed of several forms which differ in both size and charge. In addition, TnI has been resolved into a major doublet [27]. Since our results demonstrate that the TnI and TnT subunits could be separated into various components using HPLC techniques, we decided to compare these isolated components with the subunits of native troponin from the cardiac and skeletal sources. In this way it would be possible to determine whether this heterogeneity we observe in our HPLC results is similar to that previously reported.

Fig. 6 shows the two-dimensional gels of TnI, fractions I1 and I2, from both bovine cardiac and rabbit skeletal troponin. The isoforms on the two-dimensional gels are identical in size but differ in charge. The similarity in molecular weight was also observed on urea SDS-PAGE (Fig. 3). In the two-dimensional gels of TnI components a single purified component of TnT (bovine cardiac T2) was used as a standard marker. The two-dimensional gels of TnT (data not shown) isoforms indicated similarity in molecular weight (see also Fig. 3). The difference in charge of the TnT components was much less than that for TnI. This can be seen in Fig. 6 for the two-dimensional gels of native bovine cardiac troponin T and native rabbit skeletal TnT. The bovine cardiac TnT components (T1, T2 and T3) all showed single spots on two-dimensional gels as shown for bovine cardiac T2. In contrast, the rabbit skeletal TnT components from HPLC still showed multiple spots of differing charge on two-dimensional gels. Though each TnT-HPLC fraction contained more than one component, the charge of the fraction varied systematically with retention time on the ion-exchanger. These results suggested that the resolving power of two-dimensional gel electrophoresis was greater than the ion-exchange HPLC separation in this case.

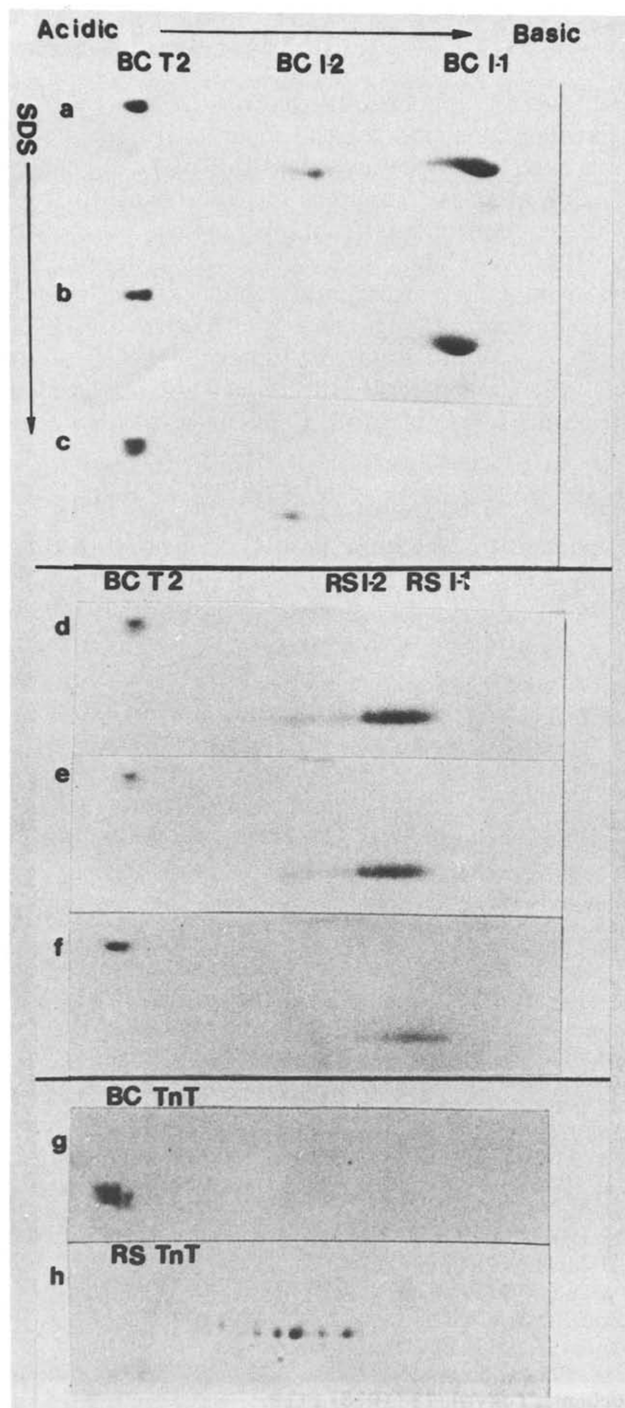

Fig. 6. Separation of bovine cardiac (BC) and rabbit skeletal (RS) troponin T and troponin I on two-dimensional PAGE. In panels a–f, HPLC-purified bovine cardiac troponin T fraction, T2, was used as a standard marker. (a) mixture of HPLC-purified BC I1 and BC I2; (b) BC I1; (c) BC I2; (d) mixture of HPLC-purified RS I1 and RS I2; (e) RS I1; (f) RS I2; (g) native bovine cardiac troponin T; (h) native rabbit skeletal troponin T.

CONCLUSIONS

From the results presented, several conclusions can be drawn: (1) Ion-exchange HPLC provides an excellent method for the separation of troponin subunits from bovine and rabbit skeletal troponin. Quantities of 10 mg can be readily purified on analytical columns. (2) Troponin T is resolved into three components: T1, T2 and T3. These components from both muscle sources are homogeneous and distinct by HPLC and urea SDS-PAGE. (3) Troponin I is resolved into two components from both cardiac and rabbit skeletal muscle troponin (I1 and I2). These fractions are homogeneous and distinct by HPLC and urea SDS-PAGE. (4) There is no indication on ion-exchange HPLC of multiple forms of troponin C. (5) Considerable differences are observed in the ionic properties of the troponin subunits from different muscle sources.

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