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SEPARATION OF THE FLUORESCENT COMPOUNDS OF BOVINE LIGAMENTUM NUCHAE ELASTIN USING SEPHADEX G-10, CELLULOSE PHOSPHATE AND THIN-LAYER CHROMATOGRAPHY

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SUMMARY

Adsorption chromatography on Sephadex G-10, ion-exchange chromatography on cellulose phosphate and thin-layer chromatography on silica gel developed with *n*-propanol–ammonia effectively separated compounds with visible fluorescence from acid-hydrolyzed bovine ligamentum nuchae elastin. These methods are compatible for use with samples containing residual hydrochloric acid. The number of fluorophores resolved were 5, 6–9 and 9 for the respective methods. Two fluorophores predominated in the cellulose phosphate and Sephadex G-10 chromatograms; the excitation/emission maxima were 338/400 and 297/394 nm and 336/398 and 295/393 nm for the respective chromatograms. Cationic charges of 0 and 3 were estimated for the 338/400 and 297/394 nm fluorophores, respectively, from cellulose phosphate chromatography. DEAE-cellulose chromatography indicated an anionic charge of 2 for the fluorophore with initial 298/393 nm spectrum. The two dominant fluorophores degraded following thin-layer chromatography indicating that the method has only qualitative use. Sephadex G-10 should prove useful for detecting changes in the amounts of the elastin fluorophores that result from degradation of or specific chemical modification of the fluorophores. The dominant fluorophores isolated by Sephadex G-10 chromatography can be used directly for chemical modification investigations.

INTRODUCTION

Methods that have been investigated for usefulness in the separation of the fluorophores of elastin include resin ion exchangers[1], adsorption chromatography using alumina[2], paper[1,3] and thin-layer chromatography (TLC)[3], Bio-Gel P-2[4] and SE-Sephadex C-25 ion exchange[4].

Resin ion exchangers are not suitable for use with elastin fluorophores. LaBella[5] noted that not all fluorophores and chromophores elute from the columns. Some are apparently either irreversibly bound or degraded by exposure to the resin. Thornhill *et al.*[1,2] reported that resins bleed a fluorescent impurity and the elastin fluorophores streak through a large portion of the chromatogram due to adsorptive interactions with the resin. Streaking of the fluorophores is a problem with paper chromato-

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graphy[1,3] and TLC[3]. Alumina is unsatisfactory for separation of the fluorophores as some components are labile under conditions of the chromatography[2].

At present the best materials for chromatography of elastin fluorophores are Bio-Gel P-2 and SE-Sephadex C-25[4]. Chromatograms obtained using Bio-Gel P-2 and SE-Sephadex C-25 show multiple fluorophores in hydrolyzed elastin. Of these two materials, SE-Sephadex C-25 seems to give better resolution of the fluorophores than does Bio-Gel P-2.

In this investigation, three new methods for chromatographing the fluorophores of elastin will be presented. Adsorption chromatography on Sephadex G-10 resulted in better resolution of the fluorescent compounds than did Bio-Gel P-2, and cellulose phosphate ion exchange gave results comparable to those reported for SE-Sephadex C-25. Cellulose phosphate is preferable to SE-Sephadex C-25 when the chromatographed hydrolysates contain substantial amounts of residual hydrochloric acid. Finally, TLC of hydrolyzed elastin on silica gel resolved nine fluorescent compounds when *n*-propanol–ammonia (70:30) was used as the developer.

EXPERIMENTAL

Materials

Bovine ligamentum nuchae elastin (E-1625) and cellulose phosphate, medium mesh, were purchased from Sigma (St. Louis, MO, U.S.A.). Bio-Gel P-2 and DEAE cellulose (Cellex-D) were purchased from Bio-Rad Labs. (Richmond, CA, U.S.A.). Sephadex G-10, sulphoethyl(SE)-Sephadex C-25 and Sephadex LH-20 were obtained from Pharmacia (Piscataway, NJ, U.S.A.) Quanta/Gram Q-1 5 × 20 cm silica gel thin-layer plates were from Quantum Industries (Fairfield, NJ, U.S.A.). All chemicals were reagent grade with the exception of *n*-propanol and *n*-butanol, which were glass distilled (Burdick and Jackson Labs., Muskegon, MI, U.S.A.).

Hydrolysis of elastin

Elastin powder (5 g) was hydrolyzed by reflux under nitrogen for 72 h in 1 l of 6 *N* HCl with 10^{-3}% $SnCl_2$. A temperature of 55°C was used for the first 30 min or until the elastin powder had completely solubilized, then the temperature was raised to 110°C. HCl was removed by rotary evaporation of successive 250-ml aliquots to a volume not less than 15 ml. No aliquot was allowed to evaporate to dryness. The final volume of each hydrolysate was adjusted to 20 ml with deionized water to give an elastin concentration of 250 mg/ml. All samples were filtered through glass wool.

Fluorescence measurements

Fluorescence measurements were made using an Aminco-Bowman spectrophotofluorometer equipped with a ratiophotometer (American Instrument, Silver Spring, MD, U.S.A.). Slits 3, 4 and 6 were 3, 1 and 3 mm. For all measurements, the sensitivity vernier was set at 100 and the sensitivity setting was adjusted as required. The fluorimeter was calibrated with quinine sulfate (1 μg/ml 0.1 *N* H_2SO_4). The relative fluorescence of the quinine sulfate standard at 350/450 nm was 55 at a sensitivity setting of 10. Wavelength calibration was periodically verified with quinine sulfate standards. Spectra were recorded with a Houston Instruments Series 2000 omnigraphic x-y recorder (Bellaire, TX, U.S.A.). All spectra reported are not further

corrected. Thin-layer plates were viewed fluorimetrically using a Chromato-Vue (Ultraviolet Products, San Gabriel, CA, U.S.A.) light box under the longer-wavelength ultraviolet lamp.

Bio-Gel P-2 chromatography

New Bio-Gel P-2 was washed extensively with 0.2 *M* acetic acid until a constant fluorescence reading at 320/400 nm (excitation/emission) was obtained. The gel was poured to a packed height of 82–86 cm in a 90 × 1.5 cm column fitted with PTFE tubing. The column was washed with 0.2 *M* acetic acid for a minimum of 24 h before use. A 1-ml sample was applied to the column in each chromatography and was eluted with 0.2 *M* acetic acid. Fractions of 1.5–2 ml were collected at 10 to 20-min intervals. Alternate fractions from the chromatogram were monitored for fluorescence at 320/400 nm.

Sephadex G-10 chromatography

Sephadex G-10 was prepared and samples were chromatographed by the same methods used for Bio-Gel P-2 chromatography. Alternate fractions were monitored for fluorescence at 320/400 and 280/310 nm. Absorbance of alternate fractions was monitored at 280 nm. Amino acids were detected by a modified ninhydrin method of Moore and Stein[6] using 250 μl of sample, 500 μl of water, 250 μl of the ninhydrin reagent and a final addition of 4 ml of 50% *n*-propanol.

Sephadex LH-20 chromatography

Sephadex LH-20 was washed with glass-distilled water. The adsorbant was poured into a 36 × 1.5 cm column to a final packed height of 32 cm. A sample of the elastin fluorophore corresponding to peak VIII in the Sephadex G-10 chromatogram was concentrated by rotary evaporation and chromatographed with glass-distilled water. Fractions of 1.5 ml were collected at 10-min intervals, and fluorescence was determined on alternate fractions at 295/393 nm.

Cellulose phosphate chromatography

Cellulose phosphate was washed with acetic acid–2 *M* NaCl (50:50) until a colorless supernatant resulted. The cellulose phosphate was then washed with 0.2 *M* acetic acid and poured into a 32 × 1.5 cm column to a packed height of 24–28 cm. Washing of the column was continued until a constant fluorescence baseline was achieved.

A 0.5-ml sample of the hydrolysate concentrated by rotary evaporation was chromatographed using a linear salt gradient (0–0.5 *M* NaCl) in a 500-ml volume of 0.2 *M* acetic acid. Fractions of 2–3 ml were collected at 10-min intervals. The chromatogram was monitored by measuring alternate fractions for fluorescence at 320/400 nm and absorbance at 280 nm. Individual spectra were taken of fractions located at the peak of fluorescence.

To detect compounds that did not bind to the cellulose phosphate, 1 ml of concentrated hydrolysate was added to cellulose phosphate and eluted with 0.2 *M* acetic acid. The non-binding fluorescent compounds were collected, concentrated by rotary evaporation to 1–2 ml and chromatographed on Sephadex G-10 as described above.

DEAE-cellulose chromatography

The Cellex-D was washed with 0.02 *M* sodium phosphate buffer, pH 8.0, until a constant fluorescence baseline was achieved. The washed ion-exchanger was poured into a 36 × 1.5 cm column to a final packed height of 34 cm. A sample of the elastin fluorophore corresponding to peak VIII in the Sephadex G-10 chromatogram was concentrated by rotary evaporation. To this sample was added an aliquot of dityrosine[7] with fluorescence intensity equal to that of the elastin fluorophore. Chromatography of the sample was done using a linear 0–0.5 *M* NaCl gradient in a 500-ml volume of 0.02 *M* sodium phosphate buffer, pH 8.0. Fractions of 1.5 ml were collected at 10-min intervals. Alternate fractions were evaluated for fluorescence intensity at 295/390 nm. Spectra were determined for samples corresponding to the peaks in the chromatogram.

Thin-layer chromatography

A 50-μl sample of the concentrated hydrolysate was streaked partially across the bottom of the plate. Sample was applied until visible fluorescence could be easily discerned when viewed under ultraviolet light. The plate was chromatographed using *n*-propanol–ammonia (70:30, v/v). The positions of the fluorescent compounds were marked while the plate was still wet.

A sample of the compound corresponding to peak IX in the Sephadex G-10 chromatogram was concentrated and applied to a thin-layer plate. Two separate development systems were used to evaluate this fluorescent compound: *n*-butanol–acetic acid–water (4:1:1, v/v/v) and *n*-propanol–ammonia (70:30, v/v). A sample of the fluorescent compound corresponding to peak XIII from cellulose phosphate chromatography was concentrated, applied to a thin-layer plate, developed with *n*-propanol–ammonia (70:30, v/v) and evaluated. When the plates were dry, they were sprayed with ninhydrin reagent[8], the ninhydrin-staining compounds were marked, and their positions were compared to those of the fluorescent compounds.

RESULTS

Bio-Gel P-2 chromatography

The chromatogram obtained by Bio-Gel P-2 chromatography of the concentrated bovine ligamentum nuchae elastin sample (Fig. 1) is similar to the Bio-Gel P-2 chromatogram reported by Thornhill[4]. However, in the referenced chromatogram, the sizes of peaks I and II were larger and the space between peaks II and III was greater than for the corresponding peaks resulting from Bio-Gel P-2 chromatography of the elastin sample evaluated in the current investigation. The fluorescence excitation and emission maxima of each peak were also different in these two studies. Peaks I and II in Thornhill's[4] investigation had maxima at 350/435 nm and peaks III and IV had maxima of 340/425 nm. In contrast, the products observed in the corresponding portions of the chromatogram in the present study had excitation maxima of 334–344 nm and emission maxima of 409–417 nm. Furthermore, the dominant fluorescence in the region corresponding to peak III had maximal fluorescence at 338/396 nm.

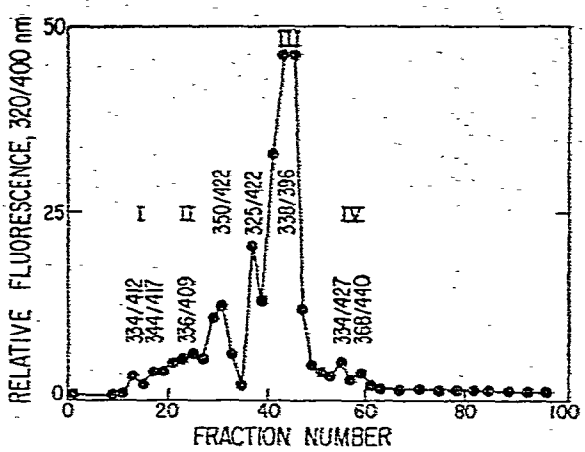

Fig. 1. Bio-Gel P-2 chromatogram of elastin hydrolysate. The areas of the chromatogram labeled I–IV are analogous to the areas of the Bio-Gel P-2 chromatogram presented by Thornhill[4] that contained fluorescent peaks given these numbers. Spectral values indicated are wavelengths at which maximal fluorescence occurred. The sensitivity setting of the fluorimeter was 30.

Sephadex G-10 chromatography

The chromatogram in Fig. 2 shows that Sephadex G-10 is an effective tool for the separation of the fluorescent compounds of elastin. Five fluorophores were resolved from the hydrolyzed elastin, and characteristic spectra for each peak are given in the figure.

A high 280-nm absorbance was associated with all the fluorophores except

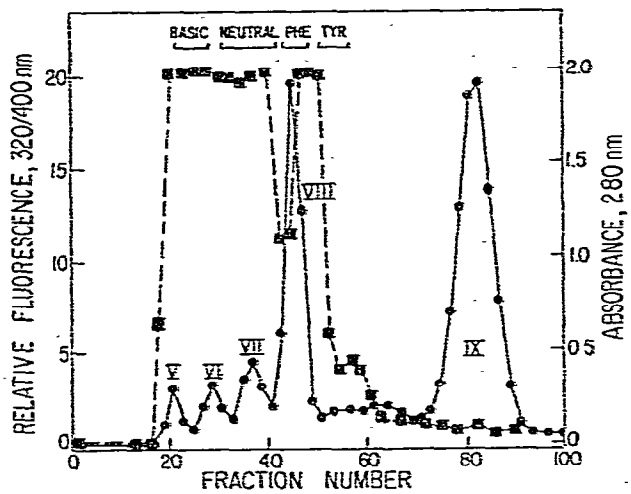

Fig. 2. Sephadex G-10 chromatogram of elastin hydrolysate. Numbering of the fluorescent peaks is an arbitrary continuation of the numbering sequence begun in the Bio-Gel P-2 chromatogram. Elution positions of the non-fluorescent amino acids are indicated above the chromatogram. Spectral maxima for the respective peaks were as follows: 362/440, V; 352/432, VI; 330/422, VII; 304/394, VIII; and 336/398, IX. (●) Fluorescence at 320/400 nm and (■) absorbance at 280 nm. The sensitivity setting of the fluorimeter was 100.

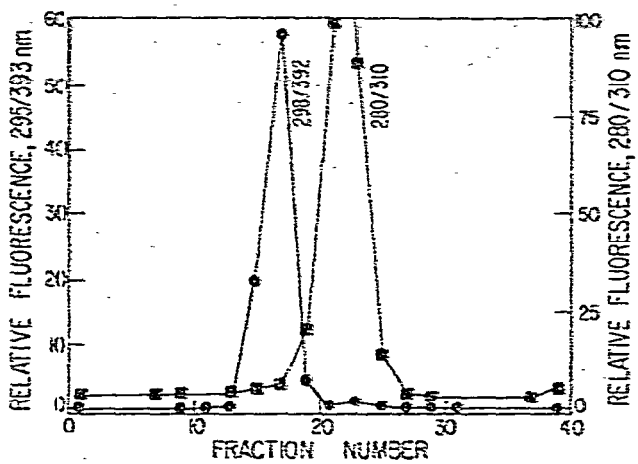

Fig. 3. Sephadex LH-20 chromatogram of the fluorophore corresponding to peak VIII in the Sephadex G-10 chromatogram. (●) Fluorescence at 295/393 nm at a sensitivity setting of 10. (■) Fluorescence at 280/310 nm at a sensitivity setting of 3.

peak IX. This absorbance led to concern that the apparent fluorescence spectra of these fluorophores were influenced by quenching. Analysis of the relative intensity of fluorescence of the pooled sample corresponding to the tyrosine peak confirmed that substantial quenching effects were present. Of the original 125,000 relative intensity units applied to the column, only 13,600 units appeared to have been recovered after chromatography. The effects of quenching on the fluorophore in peak VIII (fraction 45) were evaluated. Dilution of the sample shifted the spectral maxima from 304/394 nm to 295/393 nm. Subsequent Sephadex LH-20 chromatography of the fluorophore in peak VIII resolved the fluorophore from tyrosine (Fig. 3). The fluorescence spectra

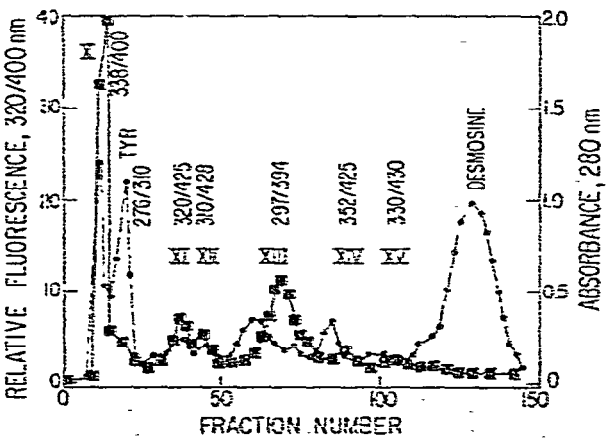

Fig. 4. Cellulose phosphate chromatogram of elastin hydrolysate. Numbering of fluorescent peaks is an arbitrary continuation of the numbering in Fig. 2. (■) Fluorescence at 320/400 nm using a sensitivity setting of 30. (●) Absorbance at 280 nm. Spectral wavelengths are those at which maximal fluorescence was obtained.

of the compound after Sephadex LH-20 chromatography was 298/392 nm, essentially identical with the fluorescence of the compound that results upon dilution of the sample obtained from Sephadex G-10 chromatography.

Cellulose phosphate chromatography

The chromatogram in Fig. 4 shows that cellulose phosphate resolved between 6 and 9 compounds with visible fluorescence. With the exception of the lack of fluorophores eluting in the area of the chromatogram around desmosine, the cellulose phosphate chromatogram is very similar to the SE-Sephadex C-25 chromatogram reported by Thornhill[4]. Compounds with absorbance at 280 nm were resolved from most of the fluorophores in the cellulose phosphate chromatogram, yet proportions of the peak fluorescence were similar to those seen in the Sephadex G-10 chromatogram. This indicated that the quenching of fluorescence that occurred in the Sephadex G-10 peaks due to compounds with absorbance at 280 nm did not significantly distort the relative proportions of the fluorophores.

Spectral maxima for the respective fluorophores confirmed that the dominant fluorophores from this chromatogram had essentially the same spectra as the dominant fluorophores resolved by Sephadex G-10. The other fluorophores displayed spectral differences that may be due to the absence of quenching by the compounds with high 280 nm absorbance.

Sephadex G-10 chromatography of the elastin fluorophore that did not bind to cellulose phosphate, corresponding to peak X of the cellulose phosphate chromatogram, revealed that several non-cationic elastin fluorophores contributed to the fluorescence of this peak (Fig. 5).

Fig. 6 displays the result of DEAE-cellulose chromatography of the compound corresponding to peak VIII in the Sephadex G-10 chromatogram. The elastin fluorophore eluted between tyrosine and dityrosine in the position appropriate for a compound with 2 anionic groups. No fluorophore was detected in the tyrosine peak sample (276/310 nm). The apparent visible fluorescence associated with this sample is

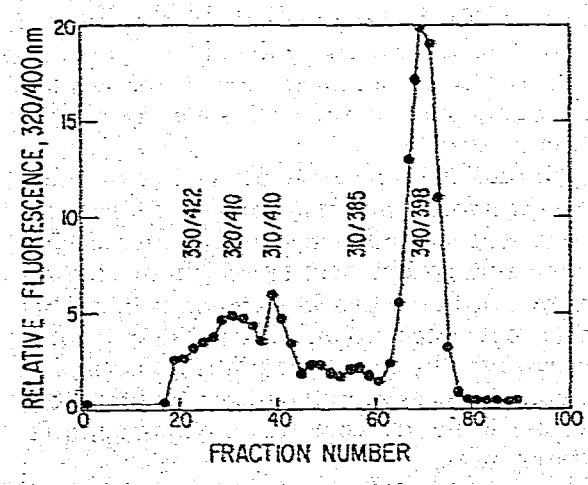

Fig. 5. Sephadex G-10 chromatogram of elastin fluorophores that did not bind to cellulose phosphate. The sensitivity setting of the fluorimeter was 100.

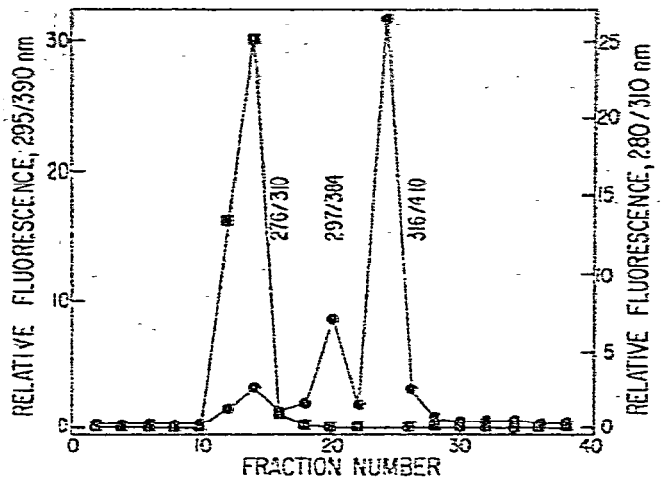

Fig. 6. DEAE-cellulose chromatogram of the fluorophore corresponding to peak VIII in the Sephadex G-10 chromatogram. (●) Fluorescence at 295/390 nm at a sensitivity setting of 10. (■) Fluorescence at 280/310 nm at a sensitivity setting of 100.

presumed to be due to tailing of the tyrosine fluorescence into the wavelengths used to monitor visible fluorescence. This is possible since the amount of tyrosine fluorescence was very large as compared to the amount of visible fluorescence being measured. Only 316/410 nm fluorescence, appropriate for dityrosine, was detected in the third peak. The decrease in fluorescence intensity of the elastin fluorophore may be due to degradation as a shift in the emission maximum to 384 nm was noted.

Thin-layer chromatography

The *n*-propanol–ammonia TLC system resolved nine distinct fluorescent bands from the hydrolysate sample (Fig. 7). These results show that it is possible to separate the elastin fluorophores from a hydrolysate by TLC without preliminary treatment of the sample. Detection of all the fluorophores required viewing the plates while they were still wet because the fluorophore with deep blue color and highest R_F rapidly lost fluorescence as the plate dried. Resaturating the plate did not reinstate this fluorescence, which indicated that decomposition occurred.

TLC of the sample corresponding to peak X in the Sephadex G-10 chromatogram gave an R_F of 50 with the *n*-propanol–ammonia solvent system and 51 using the *n*-butanol–acetic acid–water solvent system. The lack of change in migration despite the large change in pH suggests that no ionizing groups were present in this fluorophore. Detection with ninhydrin reagent revealed a ninhydrin-staining product that overlapped the fluorescent band in both solvent systems. This occurred despite other evidence showing that no amine groups are normally detectable in the fluorophore[9]. Most likely, the ninhydrin-positive compound arose as a result of degradation following the chromatography as a rapid decrease in fluorescence was obvious upon removal of the plate from the chromatography tank.

TLC of the sample corresponding to peak XIII in the cellulose phosphate chromatogram was also affected by degradation. Fig. 7B shows the resolution of

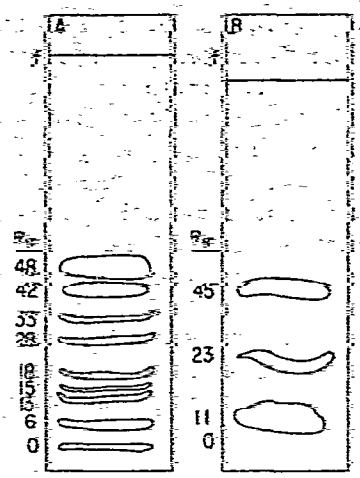

Fig. 7. (A) Silica gel thin-layer chromatogram of the elastin hydrolysate after development with *n*-propanol–ammonia (70:30, v/v). (B) Silica gel thin-layer chromatogram of the fluorophore corresponding to peak XIII in the cellulose phosphate chromatogram. The solvent was *n*-propanol–ammonia (70:30, v/v). R_F values are indicated at the left; f represents the solvent front.

three separate fluorophores with differences in polarity greater than would be expected from a sample that eluted from cellulose phosphate as a single peak. No evidence of multiple fluorophores was noted in the spectrum of the original sample before TLC. At this time it is not possible to say which of the three fluorescent bands detected correlates with the undegraded form of the 297/394 nm fluorophore.

DISCUSSION

Bio-Gel P-2 chromatography

Significant differences were noted between the Bio-Gel P-2 chromatogram and that reported by Thornhill[4]. The differences in elution positions of the fluorophores can be explained by differences in column sizes used. The discrepancy in spectral values for the fluorophores may result from differences in the initial treatment of the elastin samples. Thornhill[1] observed that swelling of elastin with pyridine allowed extraction of a fluorescent compound. The fluorophore was characterized as a saturated aldehydic lipid[2]. The fluorophore was concluded to be an artifact since it could be removed from elastin without hydrolysis of the protein. In the current investigation, the elastin samples were not treated with pyridine to remove the fluorophore, and the chromatogram undoubtedly contained this compound. No spectral data have been reported for this aldehydic lipid, so it is not possible to determine if the fluorophore with spectra of 338/396 nm as seen in the Bio-Gel P-2 chromatogram of this investigation is equivalent to the compound reported by Thornhill *et al.*[1,2].

Sephadex G-10 chromatography

Chromatographic resolution of the dominant fluorophores from the minor fluorophores with Sephadex G-10 was much better than that with Bio-Gel P-2. It should be noted that the fluorophore with 338/396 nm fluorescence seen in the Bio-

Gel P-2 chromatogram was present in the Sephadex G-10 chromatogram, and it comprised a substantial portion of the total elastin visible fluorescence. Another fluorophore (304/398 nm) was apparent in the Sephadex G-10 chromatogram that was not detected in the Bio-Gel P-2 chromatogram, which illustrates the improvement in resolution that results from use of Sephadex G-10 chromatography. An additional benefit of using Sephadex G-10 is that the major fluorophores elute well after the common amino acids. In comparison, all of the fluorophores elute from Bio-Gel P-2 in the same areas as do the common amino acids[4]. Thus, Sephadex G-10 chromatography yields in a single step a more highly purified sample of the 336/398 nm fluorophore than does Bio-Gel P-2 chromatography.

Production of artifactual fluorescence during rotary evaporation can be minimized by not allowing the sample to proceed to dryness[9], which results in residual hydrochloric acid in the sample. Residual salt in the hydrolysate sample is actually beneficial with Sephadex G-10 chromatography as it enhances the adsorptive interaction of aromatic compounds with dextrans in the gel. This interaction aids in the separation of aromatic compounds such as the elastin fluorophores[10]. Thus, Sephadex G-10 is particularly well suited for use with elastin hydrolysates.

The other dominant fluorophore eluting from Sephadex G-10 (peak VIII) appears to be sufficiently resolved from other compounds with visible fluorescence that it can be used directly for characterization studies using chemical modification. Subsequent chromatography of the fluorophore on Sephadex LH-20 resolved the fluorophore from tyrosine; however, the resulting spectra were not significantly different from those obtained upon dilution of the fluorophore sample from Sephadex G-10 chromatography. Therefore, additional purification of the fluorophore may not be necessary for most fluorimetric studies of this compound.

Cellulose phosphate chromatography

In preliminary investigations, it was found that SE-Sephadex chromatography was incompatible with the method used for preparation of elastin hydrolysates as residual hydrochloric acid in the hydrolysates caused excessive shrinking of SE-Sephadex C-25 and unacceptable slowing of the flow-rate. Chromatography using cellulose phosphate gave results similar to those obtained by Thornhill[4] with SE-Sephadex C-25, yet cellulose phosphate is not affected by the residual hydrochloric acid in the sample.

A significant finding of the cellulose phosphate chromatography is the relatively high cationic charge of the fluorophore with 297/394 nm fluorescence. Since the 297/394 nm fluorophore eluted midway between tyrosine and desmosine, a cationic charge of 3 can be estimated for this fluorophore. DEAE-cellulose chromatography of this fluorophore showed the presence of 2 anionic charges. Therefore, the fluorophore with 297/394 nm spectra is amphoteric and could function as a cross-linking moiety in elastin. The spectral values for this fluorophore are much lower than those of the ampholytes detected by Thornhill[4]. However, the Bio-Gel P-2 chromatogram of the elastin hydrolysate in the present investigation failed to resolve this fluorophore, which may explain why it has not been detected previously.

The shift in emission wavelength maximum of the 297/394 nm fluorophore following DEAE-cellulose indicated degradation of the fluorophore. This spectral shift was observed frequently. All samples of this fluorophore from cellulose phos-

phate chromatography showed a change in emission maximum within 2 days after the chromatography.

The other major peak with 338/400 nm fluorescence eluted in the area of the cellulose phosphate chromatogram appropriate for non-cationic compounds. When this sample was chromatographed on Sephadex G-10, several fluorophores cochromatographed in the same peak. These fluorophores are most likely to be the fluorophores removable by pyridine extraction[1]. Presence of the fluorophore with 340/398 nm spectra in the non-cationic portion of the cellulose phosphate chromatogram adds additional support to the hypothesis that this compound was the major fluorophore removed by pyridine extraction, and hence was not present in the Bio-Gel P-2 chromatogram reported by Thornhill[4].

Thin-layer chromatography

Use of the developer *n*-propanol–ammonia with silica gel to chromatograph the elastin hydrolysate overcame streaking of the fluorophores that has been reported for other thin-layer and paper chromatographies[3,4]. However, the two purified fluorophores that were chromatographed indicated that degradation occurred under the conditions used. Therefore, this method of elastin fluorophore chromatography may be useful only for qualitative purposes. The production of additional fluorophores from the 297/394 nm fluorophore that occurred as a result of TLC is consistent with the results of the DEAE-cellulose chromatography.

Detection of a ninhydrin-reacting group upon degradation of the 336/398 nm fluorophore during TLC may provide structural information. Failure of the fluorophore to bind cellulose phosphate or to show a change in R_F with change in pH of developing solvent argues against the presence of an amine prior to the thin layer chromatography. Exposure of an amine group upon degradation of a pyridinium ring structure has been reported[11], and this may indicate that the fluorophore is a nitrogen-containing heterocyclic compound.

CONCLUSION

Sephadex G-10 is the best material for purification of the major elastin fluorophores. The resulting fluorophores are sufficiently resolved from contaminating fluorescence that they can be used directly for further characterization. Both in Sephadex G-10 and cellulose phosphate chromatography method are suitable for use with hydrolysates that contain residual hydrochloric acid. The major fluorophores resolved by Sephadex G-10 and cellulose phosphate chromatography have spectra different from those previously reported[4]. This is due partially to the ability of Sephadex G-10 to resolve one fluorophore not resolved by Bio-Gel P-2 and partially to a difference in the initial treatment of the elastin sample. The fluorophore corresponding to peak VIII in the Sephadex G-10 chromatogram and to peak XIII in the cellulose phosphate chromatogram had 3 cationic groups and 2 anionic groups, which support a possible cross-linking role for this compound in elastin. The fluorophore was highly labile. The fluorophore corresponding to peak IX in the Sephadex G-10 chromatogram and to peak X in the cellulose phosphate chromatogram lacked cationic charge, and due to a lack of change in migration with change in pH of developer in TLC, it probably also did not have a carboxylic acid structure. Upon

degradation, a ninhydrin-reacting moiety was detected, indicating that the fluorophore may be a nitrogen-containing heterocyclic compound. The significance of this fluorophore in elastin samples is not clear; however, since it makes a sizeable contribution to the visible fluorescence of elastin, it should not be dismissed as artifactual without first determining its structural properties.

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