Linderstrøm-Lang, K. (1924), *C. R. Trav. Lab. Carlsberg 15*, No. 7.
Lumry, R., and Rajender, S. (1970), *Biopolymers 9*, 1125.
McKenzie, H. A. (1967), *Advan. Protein Chem. 22*, 55.
Nemethy, G. (1963), *Biopolymers 1*, 43.
Nemethy, G. (1967), *Angew. Chem. 6*, 195.
Piez, K., Davie, E., Fold, J., and Gladner, J. (1961), *J. Biol. Chem. 236*, 2912.
Rao, M. S. N., and Kegeles, G. (1958), *J. Amer. Chem. Soc. 80*, 5724.
Rasper, J., and Kauzmann, W. (1962), *J. Amer. Chem. Soc. 84*, 1771.
Reithel, F. J. (1963), *Advan. Protein Chem. 18*, 123.
Reithel, F. J., and Sakura, J. D. (1963), *J. Phys. Chem. 67*, 2497.
Roark, D., and Yphantis, D. A. (1969), *Ann. N. Y. Acad. Sci. 164*, 245.
Schachman, H. K. (1963), *Biochemistry 2*, 887.
Schellman, J. A. (1953), *J. Phys. Chem. 57*, 472.
Schellman, J. A. (1955), *C. R. Trav. Lab. Carlsberg 29*, 223.
Scheraga, H. A. (1961), Protein Structure, New York, N. Y., Academic Press.
Scheraga, H. A. (1963), *Proteins 1*, 477.
Singer, S. J., and Campbell, D. H. (1955), *J. Amer. Chem. Soc. 77*, 3499.
Smith, C. E., and Lauffer, M. A. (1967), *Biochemistry 6*, 2457.
Steinberg, I., and Scheraga, H. A. (1963), *J. Biol. Chem. 238*, 172.
Steiner, R. F. (1953), *Arch. Biochem. Biophys. 44*, 120.
Svensson, H. (1954), *Opt. Acta 1*, 25.
Svensson, H. (1956), *Opt. Acta 3*, 164.
Tanford, C. (1961), Physical Chemistry of Macromolecules, New York, N. Y., Wiley.
Tanford, C. (1964), *Brookhaven Symp. Biol.* No. 17, *154*.
Teller, D. C., Horbett, T. A., Richards, E. G., and Schachman, H. K. (1969), *Ann. N. Y. Acad. Sci. 164*, 66.
Timasheff, S. N., and Townend, R. (1961), *J. Amer. Chem. Soc. 83*, 464.
Timasheff, S. N., Townend, R., and Mescanti, L. (1966), *J. Biol. Chem. 241*, 8163.
van Holde, K. E., Rossetti, G. R., and Dyson, R. D. (1969), *Ann. N. Y. Acad. Sci. 164*, 279.
Verwey, E. J. W., and Overbeek, J. Th. G. (1948), Theory of the Stability of Lyophobic Colloids, New York, N. Y., Elsevier.
Yphantis, D. A. (1964), *Biochemistry 3*, 297.
Zimmerman, J. K., Barlow, G. H., and Klotz, I. M. (1970), *Arch. Biochem. Biophys. 138*, 101.

# Abductin. Locus and Spectral Characteristics of a Brown, Fluorescent Chromophore*

Donald P. Thornhill

ABSTRACT: Abductin, the elastic protein of the internal hinge ligament of the scallop, exhibits both color and visible fluorescence. Neither of these spectral properties have previously been reported and they cannot be accounted for by the presently documented amino acid composition. The ultraviolet absorption spectrum is characterized by a shoulder at 280 nm which is independent of pH and unchanged after borohydride reduction, indicating that the chromophore is neither phenolic nor quinonoid. The visible absorption spectrum is characterized by a shoulder at 460 nm which disappears after borohydride reduction. Fluorescence of abductin is not observed in the native state because of strong quenching, but in dilute solution the protein exhibits a brilliant fluorescence of slightly greater intensity than that of elastin. The fluorescence spectrum is characterized by activation and emission at 380/490 nm in acid, and 360/450 nm in alkali (uncorrected). The variation with pH of the fluorescence intensity of the protein is characterized by changes in slope at pH 2 and pH 8 indicating that the fluorophore is different from that of resilin. Acid hydrolysis of abductin liberated a brown pigment that was rapidly adsorbed on charcoal, and had a fluorescence spectrum that approximated that of the intact protein. Abductin was subjected to extensive proteolysis by pronase and the digest examined by gel permeation chromatography. The colored, fluorescent, and ultraviolet-absorbing chromophores were confined to the enzyme-resistant regions of the protein molecule, which comprised 30% of the total weight. The location, in abductin, of the chromophores which may consist of one or several species, makes them possible candidates for cross-links.

The three rubber-like proteins elastin, resilin, and abductin, are insoluble in the generally accepted protein solvents. Since these proteins dissolve only in reagents that break peptide bonds, they must be cross-linked by covalent entities other than disulfide bridges. Hydrolysates of each of these proteins have yielded novel polyfunctional amino acids that are likely candidates for forming interchain cross-links in the intact proteins. The search for the cross-linking species in elastin was centered around that protein's unusual spectral characteristics, namely its color, fluorescence, and pH-independent ultraviolet absorbance, none of which could be accounted for by the then known amino acid composition. The search culminated in the isolation and characterization of the desmosine

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TABLE I: Relative Fluorescence of Proteins in the Ultraviolet (280/305 nm due to tyrosine) and Visible Region, at Optimal Wavelengths.[a]

| Protein | Ultraviolet | | Visible | | Relative Fluorescence | |
|---|---|---|---|---|---|---|
| | Activation | Emission | Activation | Emission | Ultraviolet | Visible |
| 0.1% Abductin | 280 | 305 | | | 9.2 | |
| | | | 380 | 490 | | 35 |
| 0.1% Elastin | 280 | 305 | | | 8.2 | |
| | | | 350 | 420 | | 31 |
| 0.1% Bovine serum albumin | 280 | 305 | | | 45 | |
| | | | 350 | 420 | | 0.3 |
| 0.1% Acid-soluble collagen | 280 | 305 | | | 26 | |
| | | | 350 | 420 | | 0.7 |
| 0.1% Tryptophan | | | 290 | 350 | | 420 |

[a] Tryptophan is listed for comparison. Determinations were carried out on a double-grating instrument and are uncorrected.

isomers (Thomas *et al.*, 1963), unique polyfunctional heterocyclic amino acids. In a similar fashion the characteristic fluorescence of resilin, the protein of the wing ligament of the locust, was shown to result from the presence of dityrosine, also considered to be a cross-linking candidate (Andersen, 1966).

No report has been made on the spectral characteristics of abductin, the elastic protein of the internal hinge ligament of the scallop. This protein is highly colored and brilliantly fluorescent. These optical properties cannot be accounted for by the presently documented composition of the protein. In the present investigation a spectral examination has been made of abductin, and the distribution of the chromophores within the protein molecule ascertained. The locus of the chromophores indicates that they might be associated with a cross-linking function.

## Experimental Section

*Enzymic Hydrolysis of Abductin.* The internal hinge ligament was detached from *Aequipecten* (Marine Biological Laboratory, Woods Hole, Mass.) and immersed overnight in 0.5 M acetic acid. The protein was dried over $P_2O_5$ under vacuum to yield dark brown, hard, incompressible granules. Abductin (30 mg) was finely dispersed in 1.0 ml of pH 7.5 buffer solution in a Dual homogenizer. Two buffer solutions were used: 0.05 M collidine acetate or 0.05 M sodium phosphate. The final volume was made up to 1.5 ml and contained 5% ethanol as bacteriostatic agent. Aliquots (50 $\mu$l) of a stock solution of pronase (4 mg/ml of B grade, 45,000 PU/g, supplied by Calbiochem, Los Angeles) was added to the dispersed abductin and the mixtures incubated at 50°. Further aliquots of 50 $\mu$l of pronase solution were added at the beginning and end of each day for 3 days. Aliquots of the digest were analyzed for ninhydrin-reacting material (Rosen, 1957).

Prior to the digestion of abductin, experiments had been carried out to determine the half-life of pronase under these conditions. Dyed hide powder (Azocoll, Calbiochem., Los Angeles) was used as substrate, and the release of blue dye by pronase indicated relative proteolytic activity.

*Spectra.* Finely dispersed abductin was digested overnight with pronase at 50°. The digests were desalted on a short column of P2 Bio-Gel. Phosphate and collidine buffers were used for recording ultraviolet and visible spectra, respectively. Fluorescence spectra were determined using a double-grating instrument and are uncorrected. Reductions were carried out at pH 7–8 using sodium borohydride, and excess borohydride decomposed at pH 5.

*Fluorescence Intensity.* The relative fluorescence, at optimum wavelengths, of different proteins was determined with the stated concentrations (Table I) in water.

The variation of fluorescence intensity with pH was measured in 0.5 M citric acid–disodium hydrogen phosphate buffers. pH 1 and 13 consisted solely of HCl or NaOH solution. A filter fluorometer was used as described below.

*Acid Hydrolysis.* Abductin was hydrolyzed by heating with deoxygenated 6 N HCl under nitrogen in a sealed ampoule at 110° for 24 hr, with an acid to protein ratio of 250. In a large sample (80 mg of abductin), after hydrolysis, a small quantity of dark precipitate had formed. After several days exposure to the atmosphere more precipitate had formed and the optical density of the supernatant, originally dark brown, was much reduced. The precipitate was separated by centrifugation and washed extensively with water. After drying under vacuum the infrared absorption spectrum of the dark brown residue was determined (KBr disk). A portion of the KBr disk was then dissolved in water and the fluorescence spectrum determined.

A further acid hydrolysate of abductin (20 mg) was evaporated at 4° under vacuum, and the residue dissolved in 2 ml of $H_2O$ to yield a brown solution. Half of this solution was further diluted and the extinction at 450 nm, and fluorescence intensity, determined. The other half was transferred to a centrifuge tube, diluted to 2.5 ml, and a few milligrams of charcoal was added with stirring. After standing at 0° for 5 min the mixture was centrifuged, the extinction of the colorless supernatant measured at 450 nm, and the fluorescence determined. Both fractions of the acid hydrolysate, *i.e.*, before and after treatment with charcoal, were analyzed for amino acids, fluorescent, and ultraviolet-absorbing materials, using a Technicon amino acid analyzer. The column effluent was monitored for absorption at 280 nm, and fluorescence at 380/490 nm activation/emission.

Both fractions of the acid hydrolysate were also examined by paper chromatography, as described later.

*Gel Permeation Chromatography.* P2 polyacrylamide gel (200–400 mesh; Calbiochem, Los Angeles) was packed into a column 1.2 × 150 cm. A 10-mg aliquot of the pronase digest of abductin, in collidine buffer, was evaporated to dryness over $P_2O_5$ under vacuum, redissolved in 0.3 ml of 0.5 M acetic

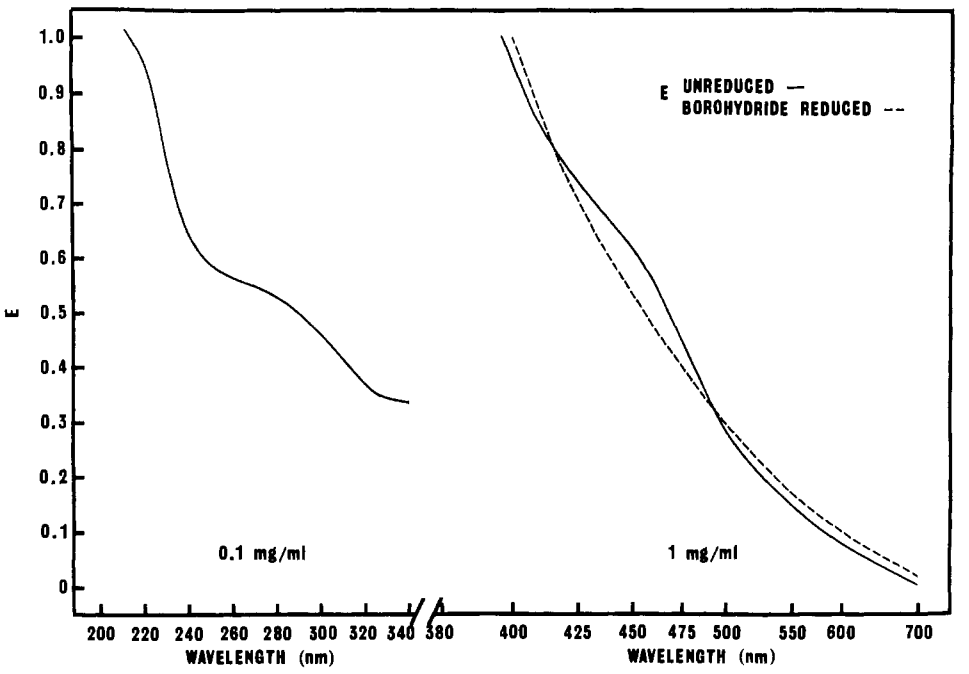


FIGURE 1: Ultraviolet and visible absorption spectra of pronase digested abductin at pH 7.4, before and after reduction with sodium borohydride. Spectra were recorded at pH 7.4, 2, and 12 without significant change.

acid, and placed on the column. Elution was carried out with 0.5 M acetic acid, and 2.0-ml fractions were collected. Each fraction was analyzed for protein (Lowry *et al.*, 1951) and ninhydrin-reacting substances (Rosen, 1957). Ultraviolet absorption was continuously monitored at 280 nm. Fluorescence was recorded with a flow cell incorporated in a filter fluorometer. Excitation filters used were a combined 360-nm dyed glass broad-range filter and a 340-nm interference filter. The emission filter was a Kodak Wratten 2B series cutoff filter transmitting beyond 400 nm.

*Paper Chromatography.* Whatman No. 1 paper was used and developed in butan-1-ol–acetic acid–water (60:15:25, v/v). Amino acids and peptides were detected with the modified ninhydrin reagent of Moffat and Lytle (1959).

## Results

*Pronase Hydrolysis.* The half-life of pronase under the conditions of the abductin digestion at 50° and pH 7.4 was 8 hr. Because of this autodigestion the pronase was added to the abductin digest in successive portions over 3 days. Dissolution of the finely dispersed abductin took place in approximately 8 hr to yield a turbid solution which eventually became clear. The course of hydrolysis, as measured by nihydrin yield, was exponential and maximal hydrolysis occurred in 48 hr.

Proteolysis in phosphate buffer was used as a control to determine if pronase was inhibited by collidine, since volatile buffers do not appear to have been used in comparable studies. There was no significant difference in the course of proteolysis carried out in either buffer.

*Absorption Spectra.* The ultraviolet and visible absorption spectra of an abductin digest are illustrated in Figure 1. This digest was yellow-orange but both the ultraviolet and visible absorption spectra (Figure 1) were devoid of distinct absorption bands. At 0.1 mg/ml and pH 7.4 a shoulder is present at 280 nm. The intensity and wavelength of this shoulder remained unaltered at pH 2 and 12, and after reduction with borohydride. At 1.0 mg/ml and pH 7.4 the visible absorption spectrum (Figure 1) is characterized by a general nonspecific absorption, with a shoulder at 460 nm. Reduction with sodium borohydride eliminated the shoulder at 460 nm (Figure 1) but the general background was unaltered. The difference spectrum at pH 1 and 14 in the visible region (Figure 2) indicated that absorption at 460 nm was largely pH independent and this spectral change, though small, followed the expected time course of reduction. The gross appearance of the protein solution did not alter.

*Fluorescence Spectra.* The fluorescence activation and emission spectra (uncorrected) of an abductin digest are illustrated in Figure 3. In alkali (pH 12) the activation and emission curves are symmetrical with maximum activation and emission at 360 and 450 nm, respectively. In acid (pH 2) the spectra are more complex and both peaks show a bathochromic shift with 40% increase in intensity. The emission peak has a well-defined maximum at 490 nm but the activation peak is complex, having one maximum intensity at 380 nm. The activation spectrum beyond 470 nm could not be determined because of scatter corresponding to the emission peak at 490 nm.

The variation with pH of visible fluorescence intensity of an enzyme digest of abductin is illustrated in Figure 4. Changes in slope appear at pH 2 and 8. This is in marked contrast to resilin (Andersen, 1966) in which the fluorescence of dityrosine was suppressed below pH 7. None of the buffers contained borate thus precluding complexing with *o,o'*-biphenols.

The effect of concentration upon the apparent fluorescence of abductin was as follows. Above a concentration of 0.1% marked quenching was evident and no appreciable fluorescence was observed at 0.4%.

The fluorescence intensity of an abductin digest is compared to other proteins and Trp, in Table I. The relative fluorescence of these materials was determined using a double-grating instrument and the data are uncorrected. It is well known that proteins are fluorescent in the ultraviolet region due to their Tyr and Trp content. Both elastin and abductin are also

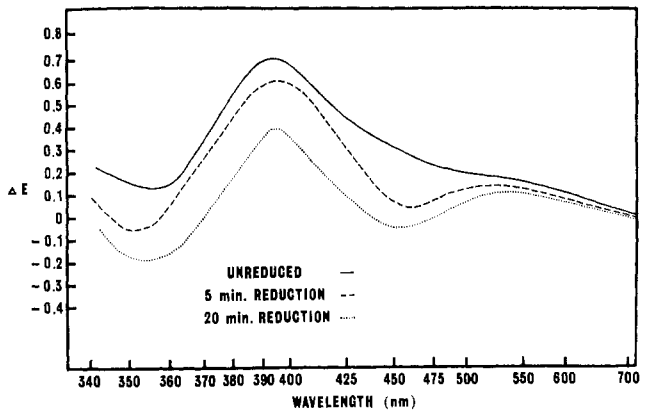


FIGURE 2: Difference spectra of unreduced and borohydride-reduced abductin digest. Digestion of abductin was carried out by pronase for 24 hr at 50°. The digest was evaporated to dryness and a solution in water adjusted to concentration = 1 mg/ml. One drop of 6 N HCl and one drop of 6 N NaOH were added to the sample and reference cells (1-cm path length), respectively. Approximately 5 mg of sodium borohydride was added to the reference cell and the difference spectra recorded after 5 and 20 min.

fluorescent in the visible region. In dilute solutions, at their respective optimum wavelengths, the fluorescence intensity of abductin is slightly greater than that of elastin.

*Products of Acid Hydrolysis.* The infrared absorption spectrum (KBr disk) of the insoluble dark brown precipitate isolated after acid hydrolysis was characterized by broad absorbance bands at 2500–3500 $cm^{-1}$ and 1000–1750 $cm^{-1}$ with a peak at 1600 $cm^{-1}$. This is consistent with the presence of NH and CO functions but no more definite conclusion may be stated. The fluorescence spectrum of a solution prepared from the KBr disk approximated that of the intact protein, and was characterized by activation per emission at 380/500 nm in acid, and 350/490 nm in alkali.

The extinction at 450 nm, of the brown solution which resulted from acid hydrolysis, was 1.75 (1-cm cell); at a concentration of 2.62 mg/ml. Charcoal treatment of the comparable fraction yielded a colorless solution of extinction 0.20. Similarly, the charcoal treatment removed most of the fluorescence.

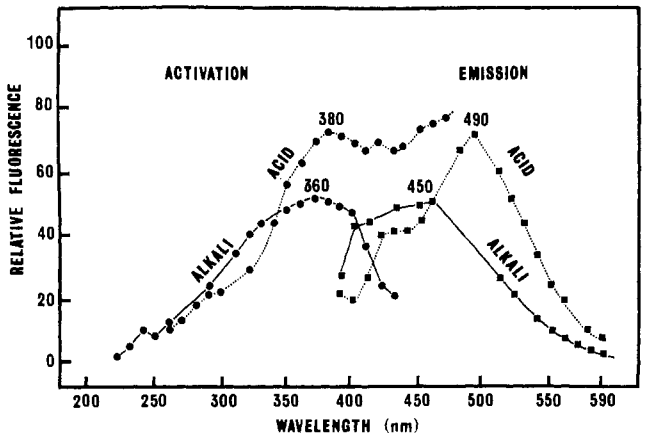


FIGURE 3: Fluorescence spectra of pronase-digested abductin. Abductin was digested for 24 hr at 50°. The digest was evaporated, redissolved in water, and adjusted to pH 2 and 12, respectively. A concentration of 0.5 mg/ml was employed in a 1-cm cylindrical quartz cuvet.

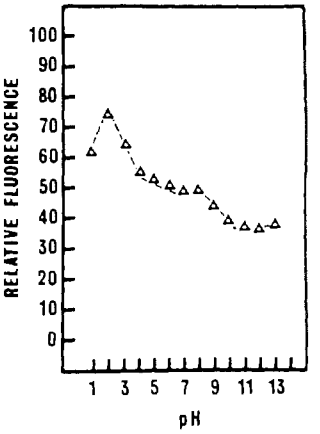


FIGURE 4: The effect of pH on the visible fluorescence of pronase-digested abductin. The pronase digest was evaporated and redissolved in water. A series of solutions (concentration 0.5 mg/ml) was made up in buffer solution with pH ranging from 1 to 13.

The amino acid compositions of the untreated (brown) and charcoal-treated (colorless) hydrolysates are shown in Table II. In neither case were definite ultraviolet (280 nm) or fluorescent (380/490 nm) peaks detected in the column effluent. However, when examined by paper chromatography the brown hydrolysate yielded a yellow-orange spot of zero mobility that was brilliantly fluorescent and stained with ninhydrin. Significantly, this spot was not present on the chromatogram derived from the charcoal-treated hydrolysate.

TABLE II: Amino Acid Composition of Acid-Hydrolyzed Abductin (Residues per Thousand) before and after Treatment with Charcoal.[a]

| Amino Acid | Untreated Hydrolysate, Brown, Fluorescent | Charcoal-Treated Hydrolysate, Colorless, Nonfluorescent |
|---|---|---|
| Asp | 16.7 | 18.4 |
| Thr | 10.4 | 11.9 |
| Ser | 62.5 | 70.2 |
| Glu | 11.0 | 12.8 |
| Pro | 8.6 | 9.7 |
| Gly | 610.8 | 634.7 |
| Ala | 70.4 | 87.8 |
| Val | 5.2 | 6.0 |
| Cys | 0.0 | 0.0 |
| Met | 81.9 | 66.5 |
| Ile | 7.9 | 6.9 |
| Leu | 2.8 | 2.1 |
| Tyr | 1.3 | 0.0 |
| Phe | 84.7 | 48.8 |
| Lys | 5.8 | 3.7 |
| His | 0.7 | 0.5 |
| Arg | 5.7 | 3.7 |

[a] The untreated hydrolysate was brown and the treated hydrolysate was colorless. Analysis was carried out on a Technicon amino acid analyzer at 60° using a sulfonated polystyrene resin. $CH_2Tyr_2$ was not resolved from phenylalanine.

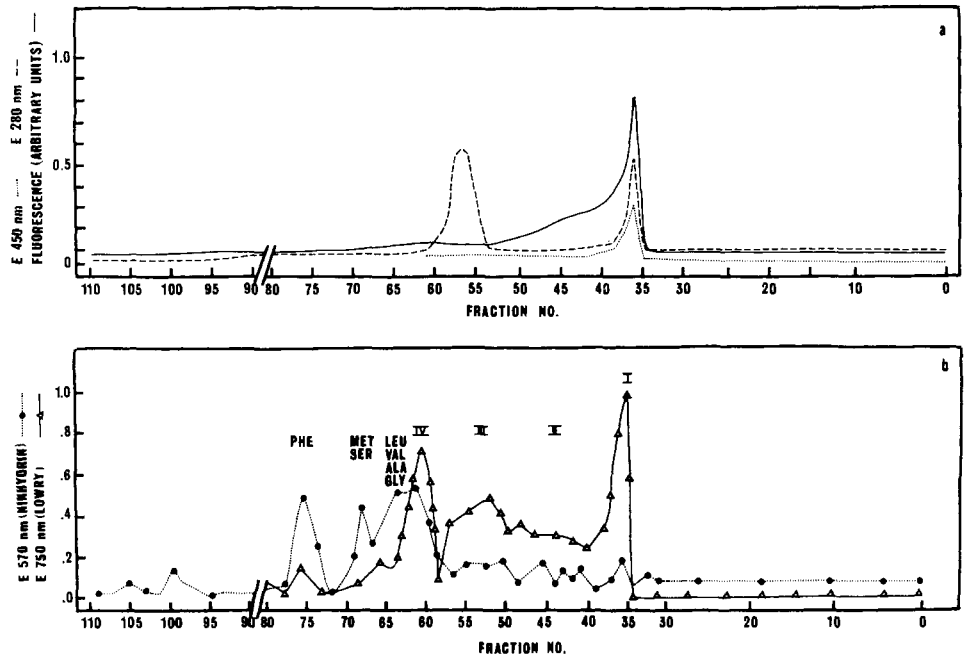


FIGURE 5: Gel permeation chromatogaphy of pronase-hydrolyzed abductin on Bio-Gel P2. A 10-mg aliquot of the pronase digest was evaporated, dissolved in 0.3 ml of 0.5 M acetic acid, and chromatographed on a 150 × 1.2 cm column of Bio-Gel P2, 200–400 mesh. Elution was carried out with 0.5 N acetic acid and 2.0-ml fractions collected. (a, top) Absorption at 280 nm, and fluorescence at 340-nm activation, 400-nm emission, were continuously recorded. Optical density at 450 nm was measured for each fraction, the plot being slightly offset to clarify the diagram. (b, bottom) Each fraction was analyzed for ninhydrin-reacting material (Rosen) and protein (Lowry).

*Gel Permeation Chromatography*. The elution profile of a pronase digest of abductin on highly cross-linked polyacrylamide gel (P2; exclusion limit, 1700) is shown in Figure 5a and 5b. The only fluorescent peak was eluted at the exclusion volume and this fraction was also yellow-orange (absorbance at 450 nm), and strongly absorbing in the ultraviolet region. After being sampled for analysis the colored fractions were evaporated to yield 3 mg of product.

The color intensities, as determined by the Lowry protein and Rosen ninhydrin procedures, are approximately inversely related, confirming the expected distribution of fragments. The greatest proportion of polypeptide fragments (area under the Lowry plot) consisted of residues of smaller molecular weight than the colored excluded fraction. All peaks were monitored by paper chromatography. The excluded fraction (peak I, fraction 36) gave a yellow-orange, fluorescent spot of zero mobility. The large peptide peak (peak IV, fraction 62) consisted of a strongly staining component, $R_F$ 0.26, and a less intense component, $R_F$ 0.46. Peptide fractions 52 and 57 (peak III) consisted mainly of one component $R_F$ 0.14. All peptide fragments stained yellow with the reagent of Moffat and Lytle (1959). Amino acid peak 64 contained Leu, Val, Ala, and a trace of Gly. Peak 68 consisted of Met and Ser and peak 75 was Phe.

The second ultraviolet-absorbing peak (fraction 57) was due to collidine (290 nm (acid) and 294 nm (alkali) with no change in $\epsilon$). This had originated in the buffer and remained associated with the protein digest even after evaporation in a relatively high vacuum (1 mm).

## Discussion

Abductin was shown by Kelly and Rice (1967) to be an amorphous extracellular elastic protein, which dissolved only in reagents that hydrolyze peptide bonds. This elasticity and insolubility imply a structure similar to classical elastomers. It is likely that abductin probably resembles elastin, *i.e.*, randomly coiled peptide chains held laterally by permanent covalent cross-links, principally of a type other than disulfide bridges.

In a search for cross-links, Kelly and Rice (1967) did not detect any unusual components in acid hydrolysates of abductin. They suggested that a material of unknown composition may have remained tightly bound to the ion-exchange column which was of a sulfonated polystyrene type. This temperature-dependent affinity of such a matrix for aromatic substances is well known and was utilized to characterize dityrosine ($Tyr_2$) in fetal elastin (LaBella *et al.*, 1967). Substances of pronounced aromatic character are very tightly bound to such resins and may not be eluted under ordinary experimental conditions. Andersen (1967) characterized $CH_2Tyr_2$,[1] a potential cross-linking residue in abductin. In contrast to $Tyr_2$, the highly colored fluorescent biphenyl structure present in resilin (Andersen, 1966), the methylene bridge in $CH_2Tyr_2$ will prevent conjugation between the aromatic nuclei and hence genesis of color in this molecule. This amino acid, therefore, is not responsible for the pigmentation of abductin.

The presence of color and fluorescence in abductin has not previously been reported. The native protein is dark brown and is not visibly fluorescent, in contrast to elastin which is characterized by a brilliant blue-white fluorescence (Partridge, 1962). In dilute solution, however (Table II), abductin fluorescence in the visible region is slightly more intense than that of elastin. The presently documented amino acid composition of abductin cannot account for fluorescence or color.

The presence of an ultraviolet-absorbing chromophore in

[1] Abbreviation used is: $CH_2Tyr_2$, 3,3′ methylenebistyrosine.

abductin is also significant. In general, strong ultraviolet absorption in the 280-nm range is usually attributable to a conjugated structure of a benzenoid or heterocyclic type, *e.g.*, the phenolic structures Tyr, $Tyr_2$, $CH_2Tyr_2$, and the nonphenolic structures Phe, Trp, and the desmosine isomers. Abductin contains very little Tyr (Table II) and the amount of $CH_2Tyr_2$ present has not previously been determined. The amount of $CH_2Tyr_2$ ($\lambda_{max}$ 278 nm (acid) and 298 nm (alkali), Andersen 1967) in abductin was not determined in the experiments reported here, as this amino acid was unresolved from the very large Phe peak in the elution system used. However, paper chromatography of an acid hydrolysate of abductin, and of authentic $CH_2Tyr_2$, indicated that very little is present. Furthermore, a significant concentration of $CH_2Tyr_2$ would be noticeable as a pH shift in the ultraviolet absorption spectrum. This is not observed. The relatively large amount of Phe in the hydrolysate cannot account for this absorbtion as $\lambda_{max}$ Phe is 260 nm and $\epsilon$ is only 200 (Wetlaufer, 1962). Trp and the desmosines are also not present (Kelly and Rice, 1967). The evidence therefore points to the presence of a hitherto undocumented ultraviolet-absorbing chromophore, almost certainly of aromatic nature but not phenolic. The presence of intense fluorescence, also normally characteristic of conjugated cyclic structures, is further evidence for the presence of aromatic components.

The brown color of the protein is apparently due to a chromophore generally absorbing between 400 and 500 nm and characterized by a shoulder at 460 nm. Because of a very strong background absorption, no definite characteristics of this chromophore can be ascertained other than partial reducibility with borohydride. Reduction does not affect the gross color of the protein solution, indicating an absence of quinonoid structures. That a brown pigment, isolated from an oxidized acid hydrolysate, had a fluorescence spectrum that approximated that of the intact protein would indicate that related species might account for both fluorescence and color. Rapid adsorption of both the brown and fluorescent chromophores onto charcoal also indicates aromaticity, but the lack of a marked variation of fluorescence intensity with pH would indicate, however, that fluorescence is not associated with phenolic structures.

The results of the fractionation of a Pronase digest of abductin on a gel column clearly indicate that the fluorescent, colored, and ultraviolet-absorbing chromophores are located at the enzyme-resistant regions of the protein molecule. These fragments would resist further enzymic hydrolysis, either because of cross-linking which would hinder enzyme attack, or because they are composed of residues that are intrinsically resistant to attack by pronase. Peptides that resist hydrolysis by pronase include Gly-Gly and Gly-Pro (Nomoto *et al.*, 1960) and this may explain why only a trace amount of free Gly was observed in the digest of the present study. However, the tripeptide Gly-Gly-Gly is hydrolyzed to a dipeptide by Pronase (Nomoto *et al.*, 1960) and the extraordinary large content of glycine in abductin, therefore, would not be expected to result in products of high molecular weight. The fragments eluted at the exclusion volume, therefore, are likely to result from gross steric consequences, rather than intrinsically resistant small peptides.

The great similarity between the physical and spectral properties of abductin and elastin is noteworthy. The elasticity, insolubility, fluorescence, and unaccountable ultraviolet absorbancy of elastin, led Partridge and coworkers to search for the components responsible for these factors. After employing a sequence of enzymes and isolating a colored, fluorescent, resistant core, the so called H peptides yielded the desmosine isomers (Thomas *et al.*, 1963), ideal candidates for cross-links. As in abductin, the ultraviolet and visible chromophores, and the flurophore of elastin all reside in the pronase-resistant regions of the protein molecule (D. P. Thornhill, to be published). That the locus of all three types of chromophore is restricted to enzyme-resistant regions of the abductin molecule suggests that they might be implicated in a cross-linking function.

In the case of abductin, it is not possible to state if all of the spectral characteristics of the protein are related to one or several species. Paper chromatography of acid hydrolysates of abductin indicates that the visible and fluorescent chromophores, which both remain at the origin, may have a highly polar character, or are easily oxidized to a polymeric material, or have a pronounced affinity for the cellulose matrix typical of highly aromatic structures. Physicochemical properties of both the colored and fluorescent chromophores thus strongly indicate the presence of aromatic nuclei.

## References


Andersen, S. O. (1966), *Acta Physiol. Scand. 66*, Suppl. 263.

Andersen, S. O. (1967), *Nature (London) 216*, 1029.

Kelly, R. E., and Rice, R. B. (1967), *Science 155*, 208.

LaBella, F., Keeley, F., Vivian, S., and Thornhill, D. (1967), *Biochem. Biophys. Res. Commun. 26*, 748.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Moffat, E. D., and Lytle, R. I. (1959), *Anal. Chem. 31*, 926.

Nomoto, M., Narahashi, Y., and Murakami, M. (1960), *J. Biochem. (Tokyo) 48*, 906.

Partridge, S. M. (1962), *Advan. Protein Chem. 17*, 227.

Rosen, H. (1957), *Arch. Biochem. Biophys. 67*, 10.

Thomas, E. J., Elsden, O. F., and Partridge, S. M. (1963), *Nature (London) 200*, 651.

Wetlaufer, D. M. (1962), *Advan. Protein Chem. 17*, 303.