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DITYROSINE IN ADHESIVE FORMED BY THE SEA MUSSEL, *MYTILUS EDULIS*

D. P. DeVore and R. J. Gruebel
Battelle, Columbus Laboratories
Columbus, Ohio 43201

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SUMMARY

The sea mussel, *Mytilus edulis*, forms an adhesive substance which is an extremely stable, alkali-soluble protein complex. Hydrolysates of the adhesive were processed using ion-exchange chromatography and fluorescent fractions compared to authenic dityrosine. UV spectra in acid solutions, fluorescent spectra, and migration on thin layer chromatography indicated that the fluorescent fraction was identical to authenic dityrosine. The tyrosine complexes function to link peptide chains into a stable three-dimensional network with unique chemical properties.

INTRODUCTION

Marine mussels and other related bivalves attach themselves to littoral substrata by means of a complex device termed "byssus". The byssus of *Mytilus edulis* consists of three primary parts: the stem, the attachment threads, and the adhesive discs. The adhesive discs provide the cementing interface between the threads and the underlying surface. Studies to elucidate the mechanisms of formation and the chemical composition of this bonding substance have included morphological(1), histochemical(2,3,4), and biochemical(5,6,7,8) investigations.

Adhesive formation appears to result from the interaction of at least three exocrine secretions(1,3,4) one of which is a polyphenolic protein. The demonstration of a polyphenolic component and the extreme resistance of the adhesive to dissolution(2,9) suggests a structure composed of a three-dimensional network linked together via biphenol complexes. The biphenol, dityrosine, has been reported in resilin(10,11), elastin(12), and in collagen(13,14).

In the present investigation, evidence is reported for the natural occurrence of dityrosine in the adhesive formed by the sea mussel, *Mytilus*

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edulis. The existence of dityrosine in the adhesive helps to explain the extreme resistance of this protein composite to chemical, physical, and enzymic degradation.

MATERIALS AND METHODS

Adhesive discs were obtained from the byssus formed by *Mytilus edulis* in a laboratory seaquarium. The discs were scraped off the glass using a razor blade. Adhesive discs, free of attachment threads, were hydrolyzed by refluxing in 6N HCl for 20 hours at 120 C. The hydrolysate was evaporated to dryness, *in vacuo*, over solid NaOH.

Authenic dityrosine was prepared by the action of horseradish peroxidase and H_2O_2 on tyrosine(15). The reaction mixture was fractionated on cellulose phosphate (1.5 x 15 cm) eluted with a continuous gradient of 0-0.5 M sodium chloride in 0.2 M acetic acid. Eluant passed through an ISCO^R UV monitor. Absorbance at 280 nm was measured and recorded and 2.0 ml fractions collected. Fractions representing separate peaks were pooled. Fluorescence was measured using the Turner Model 210 Spectrofluorimeter. Fluorescent scans from 285 nm to 405 nm were taken at an excitation of 285 nm. Ultraviolet spectra of fluorescent fractions were taken, in acid, using the Beckman Acta III recording spectrophotometer. Ascending thin layer chromatography was performed using a solvent system composed of n-butanol:acetic acid:water (55:15:30). Chromatograms were sprayed with 0.2 percent ninhydrin in acetone to locate spots. Fluorescent positive spots were located by exposure of the chromatogram to ultraviolet irradiation.

The adhesive disc hydrolysate was fractionated and examined using the same procedures described for authenic dityrosine. To identify and confirm the presence of dityrosine in the adhesive disc hydrolysate, elution patterns from cellulose phosphate chromatography, ultraviolet spectral scans, fluorescence scans, and thin layer chromatograms were compared with similar determinations for authenic dityrosine.

The amount of dityrosine in the adhesive disc hydrolysate was estimated by measuring the areas under the absorption peaks of the cellulose phosphate elution pattern.

RESULTS

Cellulose phosphate chromatography indicated the presence of dityrosine in 6N HCl hydrolysates of adhesive disc material (Figure 1). Dityrosine in isolated fractions was demonstrated by measuring ultraviolet and fluorescence spectra. Both the ultraviolet scan (Figure 2) and the fluorescence scan (Figure 3) of Fraction II were identical to those for authenic dityrosine. Finally, thin layer chromatograms showed that the fluorescent compound (Fraction II) isolated from adhesive disc hydrolysates migrated as authenic dityrosine (Figure 4).

Quantitative estimation of dityrosine in the adhesive disc was performed

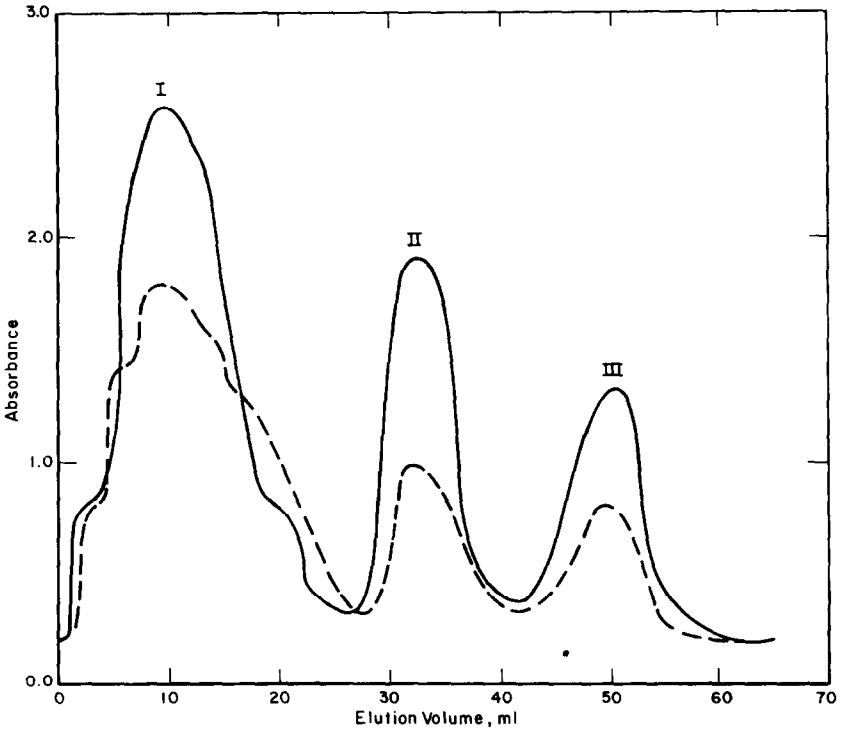

FIGURE I. FRACTIONATION OF ADHESIVE DISC HYDROLYSATE (- - -) AND AUTHENIC DITYROSINE PREPARATION (——) ON CELLULOSE PHOSPHATE EQUILIBRATED WITH 0.2 M ACETIC ACID. SAMPLES ACIDIFIED AND ELUTED WITH 0.5 M SODIUM CHLORIDE IN 0.2 M ACETIC ACID. COLUMN SIZE, 1.5 x 15 cm.

by measuring the areas under the absorption peaks of the cellulose phosphate elution patterns. The adhesive disc produced by the *Mytilus edulis* has been shown to contain 5.8 residues of tyrosine per 100 residues.(16) Other investigations have indicated as many as 8.77 residues of tyrosine per 100 residues.(17) Based on lower value and the peak areas, it is estimated that the adhesive disc contains 2.55 residues of dityrosine per 100 residues.

As noted in Figure 1, a third peak (Fraction III) was observed. This fraction corresponds to the complex, trityrosine(11) and amounted to 1.39 residues/100 residues.

The byssus thread was also analyzed for the presence of tyrosine complexes. Only a trace amount of dityrosine was indicated by cellulose phosphate chromatography.

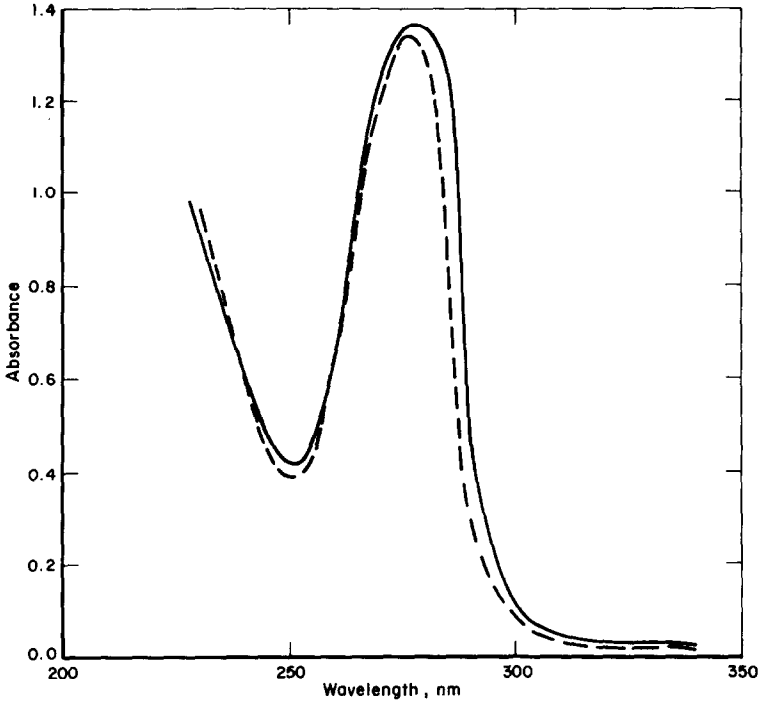

FIGURE 2. ULTRAVIOLET ABSORPTION SPECTRA FOR AUTHENIC DITYROSINE (—) AND FRACTION II OF THE ADHESIVE DISC HYDROLYSATE (---) . IN ACID SOLUTION

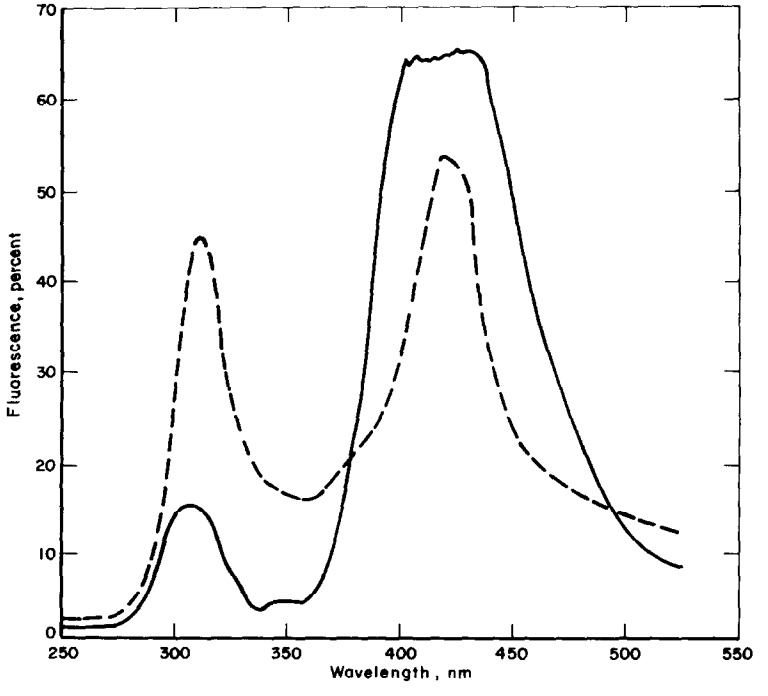

FIGURE 3. THE SPECTRA OF FLUORESCENT LIGHT FROM AUTHENIC DITYROSINE (—) AND FROM FRACTION II OF THE ADHESIVE DISC HYDROLYSATE (---) . ACTIVATION AT 285 nm

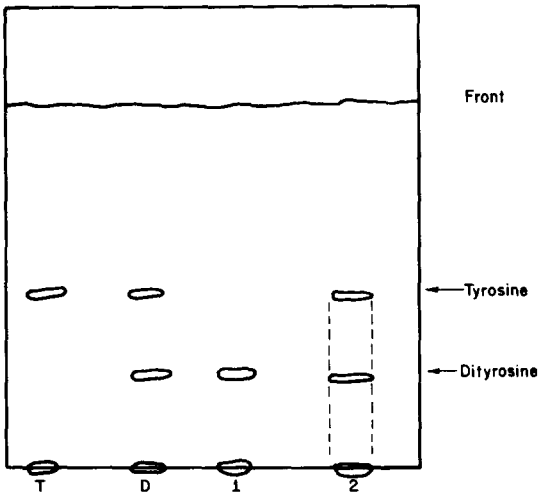

FIGURE 4. IDENTIFICATION OF DITYROSINE IN ADHESIVE DISC HYDROLYSATES. T=TYROSINE, D=AUTHENIC DITYROSINE PREPARATION 1=FRACTION OF DISC HYDROLYSATE FROM CELLULOSE PHOSPHATE CHROMATOGRAPHY, 2=DISC HYDROLYSATE: CELLULOSE SHEETS WITH FLUORESCENT INDICATOR; SOLVENT SYSTEM, n-BUTANOL-ACETIC ACID-WATER (55:15:30). SPOTS LOCATED BY NINHYDRIN AND FLUORESCENCE

DISCUSSION

Dityrosine has been demonstrated in several structural proteins(10,11,-12,13,14). The formation of dityrosine leads to a loss of solubility, induction of fluorescence, and occasionally to gel formation of protein solutions. The present investigation has demonstrated the presence of dityrosine in the adhesive substance formed by the sea mussel, *Mytilus edulis*. These amino acid complexes likely contribute to the extreme resistance of this material to dissolution.

The formation of adhesive appears to involve three and perhaps four different exocrine secretions produced by the mussel's foot.(1) One of these secretions is a tyrosine-rich protein produced by the phenol gland.(2) The concentration of tyrosine in the hardened adhesive disc is relatively high. Valves of 5.8 residues/100 residues(16), 7.7 residues/100 residues(4), and 8.77 residues/100 residues(17) have been reported. The potential of forming tyrosine complexes between protein molecules is evident. In resilin, the formation of dityrosine results in an extremely stable alkali-soluble protein.

The tyrosine concentration of resilin is only between 0.5 and 3.1 residues/100 residues and the dityrosine content of resilin has been estimated to be 1.16 residues/100 residues.(18) The adhesive formed by the *Mytilus edulis* was estimated to contain 2.55 residues of dityrosine/100 residues and 1.39 residues of trityrosine/100 residues. This concentration of tyrosine complexing would result in a highly crosslinked structure with extreme chemical stability. The sea mussel adhesive is, in fact, an alkali-soluble protein that is resistant to degradation by acids, protein solvents, detergents, alcohols, enzymes, and organic solvents.(9) This degree of stability is undoubtably due to the existence of tyrosine crosslinks between peptide chains.

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