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Mussel Glue Protein Has an Open Conformation[1]

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Both native glue protein from marine mussels and a synthetic nonhydroxylated analog were analyzed by far-uv CD under a variety of conditions. Analysis of the CD spectra using various models strongly suggests a primarily random coil structure for both forms of the protein, a fact also supported by the absence of spectral change for the glue protein upon dilution into 6 M guanidine hydrochloride. The nonhydroxylated analog, which consists of 20 repeats of the peptide sequence Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys, was further characterized by enzyme modification using mushroom tyrosinase. Enzymatic hydroxylation of tyrosines was found to be best fit by a model containing two rate constants, $5.6\ (\pm 0.6) \times 10^{-3}$ and $7.2\ (\pm 0.3) \times 10^{-2}$ min $^{-1}$. At equilibrium, HPLC analysis of digests showed nearly 100% conversion of Tyr-9 and only 15 to 35% conversion of Tyr-5. The Chou and Fasman rules for predicting structure were applied to the repeat sequence listed above. The rules predict the absence of α helix and β pleated sheets in the structure of this peptide. On the other hand, β turns are predicted to be present with Tyr-5 being in the region of highest probability. These data suggest that the protein in solution has only a small amount of secondary structure.

Most of the cells and tissues of living organisms rely on a very specific and dynamic array of molecular interactions for their cohesive integrity. In contrast, the attachment of marine mussels to a variety of surfaces ranging from paraffin to slate is remarkable for its nonspecificity and permanence (1). The polyphenolic or glue protein responsible for mussel adhesion has recently been characterized and consists largely of 75–80 tandemly repeated hexa- and decapeptides, i.e., Ala-Lys-Pro-Thr-(Tyr/Dopa[4])-Lys and Ala-Lys-Pro-Ser-(Tyr/Dopa)-Hyp-Hyp-Thr-Dopa-Lys (2). The protein adsorbs to surfaces following secretion as a semistable foam by foot of the mussel. Subsequently, the foam hardens and forms a permanent bond between the surface and the mostly collagenous byssal threads (3, 4).

Protein adsorption, the initial event in adhesion, is intricately related to the functional chemistry and conformation of the proteins. The functional chemistry of the glue protein suggests that it is capable of strong, even irreversible, adsorption to surfaces. In particular, the Dopa groups form complexes with Al(III), Fe(III), and Si(IV) with log stability constants in excess of 30 (5–7). Moreover, the hydrogen

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[4] Abbreviations used: Dopa, 3,4-dihydrophenylalanine; SDS, sodium dodecyl sulfate; PTH, phenylthiohydantoin.

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bonding of *o*-diphenols to hydrophilic polymers is competitive with that of water (8). Nothing is currently known about the conformation of mussel glue protein. In general, proteins with flexible or unordered conformations are more fully adsorbed than those with tight globular structures (9). The highly repetitive primary sequence of the glue protein, however, is reminiscent of collagen (10), silk fibroin (11), fish antifreeze protein (12), and others all of which form highly ordered, even paracrystalline, structures. The presence of such order would be difficult to reconcile with the need for extensive nonspecific adsorption.

Because of the need to better understand protein adsorption and the growing importance of mussel glue as a cell and tissue culture adhesive, we have undertaken to ascertain what, if any, conformation is present. Circular dichroism and enzymatic modification studies suggest that the mussel glue protein may have a largely unordered extended conformation in solution with little or no secondary structure.

MATERIALS AND METHODS

Materials

The naturally occurring glue protein was purified as previously described (2). A protein analog of the mussel polyphenolic protein with 216 amino acids and M_r 24,000 containing 20 repeats of the decapeptide sequence (Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys) was synthesized using established recombinant DNA methodology in *Saccharomyces cerevisiae* by GENEX Corp. (Gaithersburg, MD). The complete sequence of the protein is

	5	10	15	20	25	30
	A A A K P S Y P P T Y K^A K P S Y P P T Y K^A K P S Y P P T					
31	Y K A^K P S Y P P T Y K^A K P S Y P P T Y K^**T P A** A K P S Y					
61	P P T Y K^A K P S Y P P T Y K^A K P S Y P P T Y K^A K P S Y					
91	P P T Y K^A K P S Y P P T Y K^**T P A** A K P S Y P P T Y K^A K					
121	P S Y P P T Y K^A K P S Y P P T Y K^A K P S Y P P T Y K^A K					
151	P S Y P P T Y K^**T P A** A K P S Y P P T Y K^A K P S Y P P T Y					
181	K^A K P S Y P P T Y K^A K P S Y P P T Y K^A K P S Y P P T Y					
211	K^**T P A S S**					

where the symbols (^) denote trypsin sensitive sites, and the bold amino acids represent sequences not known to be present in the natural protein. This analog of the naturally occurring glue protein will be referred to as the 20-mer. As received from the manufacturer, the 20-mer had an apparent M_r 31,000-32,000 as determined by SDS-gel electrophoresis and was essentially pure. Other reagents and their respective suppliers were as follows: mushroom tyrosinase (sp act 4300 units/mg) and ascorbic acid (Sigma), Glyco-Gel B, a boronate affinity resin (Pierce Chemical Co), and trypsin (Boehringer-Mannheim). Ultrapure guanidine hydrochloride (Schwartz/Mann) and ultrapure sodium chloride (Alfa Products) were used in the buffers for the CD measurements.

Methods

Enzymatic hydroxylation of tyrosyl residues. The enzymatic hydroxylation of tyrosine residues in the 20-mer was carried out essentially as described previously (13). Briefly, 6 mg of 20-mer was dissolved in 10 ml of 0.1 M sodium phosphate (pH 6.8) containing 25 mM ascorbate. Mushroom tyrosinase (0.5 mg) was added, and the reaction was allowed to proceed for 3 h at 20°C with continuous aeration and stirring. The reaction was terminated by the addition of 0.1 M NaOH and distilled water to give a final pH of 8.0 and 0.05 M phosphate concentration. The sample was then applied to a boronate affinity column (3.2 × 1.0 cm) previously equilibrated with 0.05 M phosphate, pH 8.0 (14). Only Dopa-containing 20-mer was bound to this column through *cis*-diol boronate complexes, where as ascorbate and tyrosinase flushed through directly. The Dopa-containing 20-mer was eluted with 5% (v/v) acetic acid and monitored spectrophotometrically at 280 nm and by the molybdate-nitrite assay (15). Peak fractions were pooled and purified by reversed-phase HPLC using a C-8 column (Brownlee, RP-300) with two linear gradients of acetonitrile in water and 0.1% (v/v) trifluoroacetic acid: from 0 to 16% at 16 min and from 16 to 37% at 63 min using a flow rate of 1 ml/min. About 2.4 mg (40% yield) of Dopa-containing 20-mer was recovered following removal of acetonitrile and water by freeze-drying. The low yield reflects both the loss of protein due to quinone crosslinking during treatment with tyrosinase and the partial recovery of 20-mer from the affinity column.

Preparation of Dopa-containing peptides. Dopa-containing decapeptides were prepared from the 20-mer by trypsinization (2). The hydroxylated 20-mer (2 mg) was dissolved in 3 ml 0.05 M sodium borate, pH 8.0, with 1 mM $CaCl_2$. Trypsin was added at an enzyme:protein ratio of 1:100 (by weight) and digested for 12 h at 25°C under 40 psi of N_2. The sample was flash evaporated to a final volume of 1 ml, applied to a column of LH-Sephadex C-20 (1.5 × 74 cm), and eluted with 0.2 M acetic acid. Peak fractions (280 nm) were pooled and resolved by C-8 reversed-phase chromatography (Brownlee, RP-300) using the gradient system described above.

Electrophoresis. The molecular weight of the 20-mer was determined by polyacrylamide gel electrophoresis with sodium dodecyl sulfate using a 12% separating and 4% stacking gel under denaturing and reducing conditions. Slab gels were run in a Tris-glycine buffer system as described in the Hoefer Scientific Instruments catalog. Polyacrylamide gel electrophoresis was also performed in 7.5% separating gels with 5% acetic acid and 2 M urea (16).

Amino acid analysis. For amino acid analysis, proteins were hydrolyzed in 6 N HCl with 5% (v/v) phenol for 24 h *in vacuo* at 100°C. Special conditions are usually necessary to resolve leucine and Dopa by amino acid analysis (15). The sequence of tryptic peptides was determined by PTH derivatization using a Beckman 890C sequenator with 0.1 M Quadrol in the presence of polybrene and Gly-Gly-Gly (2).

CD measurements. CD measurements were made on a JASCO J-500A equipped with a DP-500 data processor and spectra were corrected for baseline. Unless stated otherwise, 5 mM molar phosphate buffer, pH 7.0, was present in all solutions to help maintain the pH. Routinely, 0.3–1.0 mg/ml samples in a 0.1-mm cell were scanned an average age of 16 accumulations with the sensitivity setting at 1.0°/cm, scan speed at 20 nm/min, and the time constant at 1 s. A value of 115.4 mean residue molecular weight was calculated from amino acid composition and was used in the conversion to mean residue ellipticity.

Estimates of structure. The mean residue ellipticity, $[\theta]$, at any wavelength, ϕ, can be expressed as

$$[\theta]_\phi = \sum f_c[\theta]_c, \qquad [1]$$

where f_c refers to the fractional contribution of a structure and $[\theta]_c$ refers to the mean residue ellipticities for that structure. Using an equation in the form of Eq. [1], estimates of the percentage of α, β, and random structures were obtained from least-squares fits of the spectra to a set of standard values determined by Chen *et al.* (17). In addition, estimates of α, β, β turn, and random structures were obtained by Bolotina (18).

RESULTS

Circular dichroism spectra of the glue protein and of the nonhydroxylated 20-mer were obtained in the far-uv wavelength range under a variety of conditions. The spectra of the glue protein in a pH 7.0 buffer containing either 0.6 M KF, 0.6 M NaCl, or 6.0 M guanidine hydrochloride are shown in Fig. 1. Due to the absorption of light by the buffer salts, all spectra could not be obtained over a full 190–250 nm wavelength range; however, comparison of the spectra over the 205–250 nm region show only minor differences that could readily be explained by error in the measurement. The spectra of the nonhydroxylated 20-mer were also obtained and found to be nearly the same as the spectra of the glue protein in buffer solutions which contained either 6.0 M guanidine hydrochloride or 0.6 M NaCl.

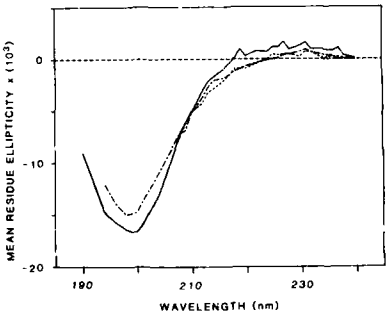

FIG. 1. CD spectra of the glue protein. Spectra of the glue protein were obtained in a 5 mM pH 7.0 phosphate buffer containing (—) 0.6 M KF, (---) 0.6 M NaCl, or (- - - -) 6.0 M guanidine-HCl. Spectra were accumulated at a temperature of 22°C as described under Materials and Methods.

Since there appeared to be little change in the spectral characteristics of the glue protein in the different solutions, the effect of temperature was examined as a means of perturbing the spectrum (Fig. 2). Temperatures of 4, 22, and 37°C were compared in the NaCl and KCl containing buffers (Figs. 2A and 2B), while 25 and 60°C were used for the 6.0 M guanidine hydrochloride containing buffer (Fig. 2C). In all three solutions, the spectrum was found to change systematically with the mean residue ellipticity becoming more negative in the

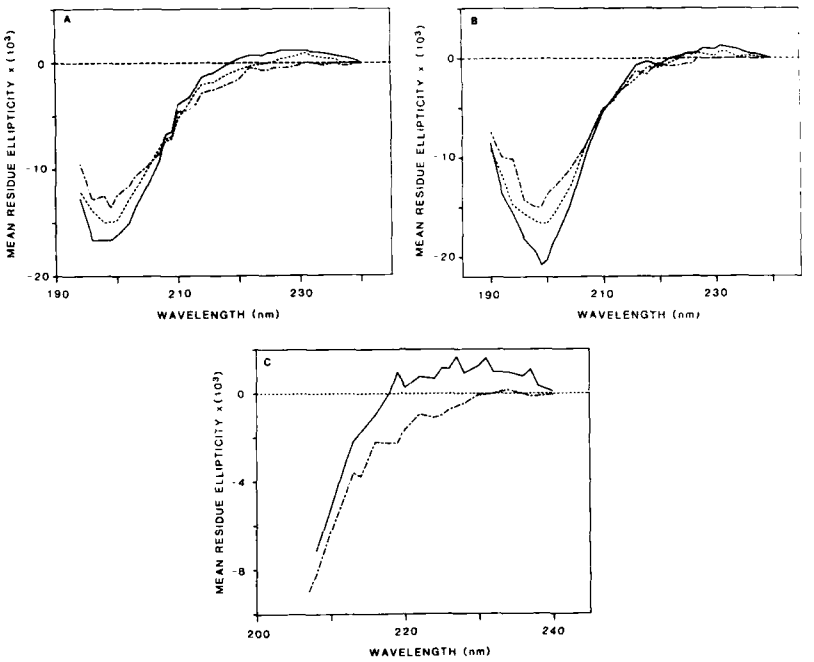

FIG. 2. (A) CD spectra of the glue protein in NaCl as a function of temperature. Spectra of the glue protein were obtained in a 5 mM phosphate buffer, pH 7.0, containing 0.6 M NaCl at temperatures of (—) 4°C, (---) 22°C, or (- - - -) 37°C. (B) CD spectra of the glue protein in KCl as a function of temperature. Spectra of the glue protein were obtained in a 5 mM phosphate buffer, pH 7.0, containing 0.6 M KCl at temperatures of (—) 4°C, (---) 22°C, or (- - - -) 37°C. (C) CD spectra of the glue protein in guanidine hydrochloride as a function of temperature. Spectra of the glue protein were obtained in 6.0 M guanidine hydrochloride at temperatures of (---) 60°C or (—) 25°C.

215-230 nm region and less negative in the 200-215 nm region as the temperature was increased.

Estimates of secondary structure were obtained by analysis of the spectra obtained under all of the various conditions. The spectral analysis was performed by least-squares fits to model data taken from Chen *et al.* (17), (Table I). Alternatively, model data of Bolotina were used (18) (Table II). The difference in these two models is the inclusion of parameters for estimates of β turns in the model of Bolotina. The results of the analysis of the protein in buffers containing NaCl and KCl at 25°C suggest that the protein is 65-75% "random" structure. Inclusion of up to 6.0 M guanidine hydrochloride, at 25°C, did not appear to alter the structure significantly as reflected in the estimates of 71 and 79% for the random structure (Tables I and II). Other spectra of the glue protein were obtained at different concentrations of NaCl, lower concentrations of guanidine hydrochloride, or in the presence of EDTA or metals. None of these alterations in the content of the solutions were found to significantly alter the spectra or the final analysis.

TABLE I

ANALYSIS USING THE VALUES OF CHEN *et al.* (17)

	α	β	Random
0.6 M KF			
37°C	4.3 ± 1.5	24.2 ± 4.5	71.5
25°C	2.1 ± 1.3	23.3 ± 4.0	74.6
22°C	2.8 ± 1.4	25.8 ± 4.2	71.4
4°C	1.1 ± 1.3	24.7 ± 4.3	74.2
0.6 M NaCl			
37°C	6.3 ± 1.8	29.6 ± 3.9	64.1
25°C	3.9 ± 0.9	26.0 ± 2.0	70.1
22°C	4.2 ± 2.0	30.9 ± 4.4	64.9
4°C	2.1 ± 2.5	28.5 ± 5.3	69.4
6.0 M GDN			
60°C	4.3 ± 0.5	21.1 ± 1.9	74.6
25°C	0.0 ± 0.7	20.3 ± 2.6	79.7
0.6 M NaCl (nonhydroxylated 20-mer)			
25°C	4.6 ± 0.1	28.9 ± 0.2	66.5
6.0 M GDN (non-hydroxylated 20-mer)			
25°C	3.2 ± 0.1	19.8 ± 0.2	77.0

The nonhydroxylated 20-mer exhibited CD spectra nearly identical to those of the natural glue protein in all buffers. Consequently we used the 20-mer as a model and evaluated its amino acid sequence by the Chou and Fasman rules of analysis in order to correlate the predicted structure to the estimated structure obtained from the analysis of the CD spectra. For the decapeptide (Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys) the analysis suggests that there is little probability of formation of any β or α structures while the four-amino acid sequences (Lys-Pro-Ser-Tyr) and (Pro-Pro-Thr-Tyr) are sequences with a good probability for forming β turns. While the Chou and Fasman (19) analysis which predicts little α and β structure is supported by the analysis of the CD spectra, the presence of only 18.6% β turn structure is detectable on the basis of the CD spectrum (Table II). Further evidence supporting the absence of secondary structure in the native protein and in the 20-mer comes from the fact that guanidine hydrochloride was unable to cause any perturbations in the spectra. Also, the similar spectra obtained for the hydroxylated and nonhydroxylated forms suggest that the degree of hydroxylation does little to influence secondary structure of the protein in solution.

Enzymatic studies were performed in order to examine the accessibility of tyrosyl groups in the 20-mer to enzymatic hydroxylation. Under reducing conditions after 3 h, mushroom tyrosinase converted about 44 ± 5% of tyrosyl residues to Dopa. This value was calculated from the ratio Dopa/(Dopa + tyrosine). In the HPLC-purified tyrosinase-treated protein, the level of hydroxylation was slightly higher at 56 ± 4% presumably due to removal of cross-linked protein and enzyme. Figure 3 shows the time course of the tyrosinase-catalyzed hydroxylation of 20-mer. The data for the appearance of normalized [Dopa/(Tyr + Dopa)] and the disappearance of normalized tyrosine [Tyr/(Dopa + Tyr)] were pooled and fit by a non-linear least-squares analysis with the percentages of the fractions from the HPLC separation used as constraints. The best fit of the data (Fig. 3) was found to be a model with two rate constants $k_1 = 5.6\ (\pm 0.3) \times 10^{-3}$ min $^{-1}$ and $7.2\ (\pm 0.3) \times 10^{-2}$ min $^{-1}$. These data suggest at least two significantly different accessibilities of tyrosyl groups to tyrosinase. To assess this possibility, tyrosinase-treated

TABLE II

CD ANALYSIS USING REFERENCE VALUES OF BOLOTINA (18)

	α	β	β Turn	Random
0.6 M NaCl				
37°C	5.9 ± 0.5	11.9 ± 0.7	18.8 ± 0.5	63.4
25°C	4.0 ± 1.0	11.7 ± 1.5	18.3 ± 1.2	66.0
22°C	2.9 ± 0.6	12.2 ± 0.8	17.8 ± 0.6	67.1
4°C	1.9 ± 0.5	11.4 ± 0.8	19.2 ± 0.5	67.5
0.6 M KF				
37°C	5.7 ± 0.6	10.5 ± 0.9	19.6 ± 0.7	64.2
25°C	4.0 ± 0.5	10.4 ± 1.0	19.8 ± 0.6	65.8
22°C	3.6 ± 0.5	11.1 ± 0.7	18.7 ± 0.5	66.6
4°C	2.5 ± 0.7	10.4 ± 0.9	18.4 ± 0.6	68.7
6.0 M GDN				
60°C	4.2 ± 0.6	11.4 ± 0.8	16.3 ± 0.8	68.1
25°C	0.0 ± 1.0	11.9 ± 1.2	16.6 ± 1.2	71.5
0.6 M NaCl (nonhydroxylated 20-mer)				
25°C	5.3 ± 0.7	15.3 ± 1.0	18.6 ± 0.7	60.8
6.0 M GDN (nonhydroxylated 20-mer)				
25°C	3.2 ± 0.1	10.1 ± 0.9	16.8 ± 0.5	69.9

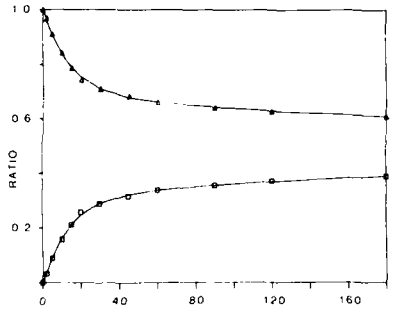

FIG. 3. Enzymatic conversion of tyrosine to Dopa by mushroom tyrosinase. The enzymatic hydroxylation of tyrosines on the 20-mer was carried out as described under Materials and Methods. The samples were collected as a function of time and quantitated by amino acid analysis of HPLC fractions obtained after trypsization. The data are expressed as a ratio of remaining tyrosine (△) or newly formed Dopa (□) at a given reaction time, to the total tyrosine of the 20-mer at time 0. The line through the points represents a fit of a biphasic reaction model to the data.

TABLE III

AMINO ACID SEQUENCE OF THREE RP-300 FRACTIONS (FIG. 4) FOLLOWING TRYPSINIZATION OF 20-MER HYDROXYLATED IN THE PRESENCE OF MUSHROOM TYROSINASE

Position	Amino acid (nmol)	Percentage of total[a]
	Fraction A	
5	Tyr (1.0); Dopa (8.2)	15
9	Dopa (4.6); Tyr (0.04)	
	Fraction B	
5	Tyr (4.6); Dopa (0.5)	60
9	Dopa (3.8); Tyr (0.2)	
	Fraction C	
5	Tyr (2.2); Dopa (0.12)	21
9	Dopa (2.1); Tyr (0.3)	

[a] Percentage of each peptide relative to the sum of the amount of all the eluted peptides.

20-mer was digested with trypsin, and a purification of the peptides was attempted by reversed-phase HPLC. The elution profile shown in Fig. 4 reflects that the tryptic peptides have very similar mobilities on HPLC and indeed could not be cleanly separated from one another on any resin. The amino acid composition of fractions contained in these peaks is listed in Table III and ranges from 90 to 55% Dopa conversion with the Dopa-richest fractions eluting earliest. Three lots of fractions were collected for sequencing to determine if there was any preference by the enzyme for Tyr-5 or Tyr-9 in the consensus repeats. Sequence results suggest essentially a quantitative conversion of Tyr-9 to Dopa and slight but perceptible conversion of Tyr-5 to Dopa, especially in the earlier eluting peaks (Table III). The Dopa-rich, early eluting fractions represent a small portion (≤15%) of the total peptides produced.

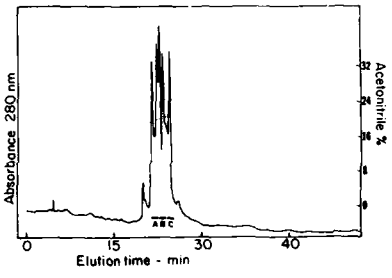

FIG. 4. Elution profile of the trypsin-digested hydroxylated synthetic mussel glue analog on a C-8 reversed-phase column. Fractions were assayed at 280 nm and molybdate-nitrite reactivity at 500 nm. Fractions under the bars marked A, B, and C were pooled for amino acid analysis and peptide sequencing.

DISCUSSION

In attempting conformational analysis from CD spectra, it should be emphasized that a number of factors can strongly affect CD spectra (20). However, as indicated by numerous literature articles, CD spectra may be used to estimate conformations present in a qualitative way, but ab-

solute numerical values should not be accepted as such (20). The data from the analysis of *far-uv CD* spectra of the polyhydroxylated form of the glue protein suggest that the protein has an unordered structure. Whether the glue protein or the 20-mer analog contain any ordered structure remains unclear. The spectra of both are reminiscent of those generally observed for highly denatured proteins (20). Furthermore, addition of 6.0 M guanidine hydrochloride, which transforms many proteins to a random coil, causes no change in the CD spectrum.

The CD data for the 20-mer were very similar to the data collected for the polyhydroxylated glue protein. Applying the rules of Chou and Fasman for predictions of structure to two repeats of a 10-amino acid segment of the 20-mer indicates that α helix or β sheets were not likely while some β turns might exist in two places. The location with the highest probability of β turns contains Tyr-5, i.e., Lys-Pro-Ser-Tyr. The second possible amino acid segment contains Tyr - 9, i.e., Pro - Pro - Thr-Tyr.

The 20-mer protein which consists of 216 amino acids in the form of about 20 repeats of a decapeptide, was designed as a synthetic analog of protopolyphenolic protein, that is, polyphenolic adhesive protein of *M. edulis* prior to any post-translational hydroxylation at tyrosine and proline. Nearly 78% of the tyrosyl groups in natural polyphenolic protein are converted to Dopa (2), and the Dopa is thought to play an essential role in the adhesive and cohesive properties of the protein by hydrogen bonding, metal chelate complexes, and covalent crosslinks. Sequence studies of tryptic peptides derived from the natural polymer have suggested that two different tyrosyl environments occur. These are both presented in the consensus repeat

Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys.

When tyrosinase is added to the 20-mer, only 15 to 35% of Tyr-5 is found hydroxylated to Dopa while essentially 100% of Tyr-9 is converted. *In vivo*, the post-translational hydroxylation is likely due to a putative tyrosyl 3-hydroxylase. Apart from the slight difference in primary structure around the two tyrosines in the repeat, there is no basis yet for concluding that the factors determining enzyme-substrate preference are ruled by primary structure, secondary structure, or a combination of the two. Prolyl 4-hydroxylase in plants appears to recognize a poly-L-proline II helix, hydroxylating both protocollagen and polyproline with similar facility (21). Prolyl 4-hydroxylase in animals, however, has very specific primary sequence requirements, i.e., $(X\text{-}Y\text{-}Gly)_n$, where Y must be Pro (22). Since tyrosyl 3-hydroxylase acting on protein-bound tyrosyl groups has yet to be isolated, nothing is known about its properties except a product (polyphenolic protein) that is a fait accompli.

Mushroom tyrosinase has a long history of use as an agent of site-directed modification. The enzyme catalyzes both the hydroxylation of monophenols such as tyrosine, including protein tyrosyl groups, and the oxidative dehydrogenation of *o*-diphenols to quinones (23). In the presence of high concentrations of ascorbate the reaction can be limited to hydroxylation since the *o*-quinone is reduced back to the *o*-diphenol by ascorbate. Mushroom tyrosinase oxidizes Tyr-26 in the B-chain of insulin at a rate 13 times faster than Tyr-16 which is thought to be buried (24). The present results suggest that there are two tyrosyl reactivities in the 20-mer analog of the polyphenolic protein. These vary in their rate of hydroxylation by a factor of about 20. The sequence of the hydroxylated peptides derived from the 20-mer by trypsinization indicated that Tyr-9 is almost completely converted to Dopa by tyrosinase, while Tyr-5 is only marginally modified in the peptides. Perhaps Tyr-9 is the more accessible of the two. This pattern of hydroxylation is similar to that of the natural protein, except that the levels of Tyr-5 to Dopa conversion at 3 h in the 20-mer are somewhat lower than those in polyphenolic protein.

The results of the tyrosine hydroxylation appear to be inconsistent with the CD results which suggest minimal structure.

The CD analyses yield values around 18% for the β turn, the only structure predicted by the Chou and Fasman analysis. However, β turn predictions from CD analysis do not always correlate as well with known protein structures as they do for α helix and β sheet (18). Therefore, NMR studies have been frequently used to enhance structural analysis of peptides. Recent NMR work of Cung *et al.* (25) suggested that a L-Pro-L-Pro containing peptide exhibits flexibility in aqueous solutions and a mix of two types of folded conformations in inert solvents. Otter *et al.* (26) concluded that a different peptide containing the Pro-Pro sequence had some type of structure that was not disrupted by 5 M urea. Therefore it is possible that Tyr-9 being close to a Pro-Pro sequence could be part of a guanidine hydrochloride stable structure that makes it more accessible to enzymatic hydroxylation, and that Tyr-5, being in a high probability region for a β turn, may be part of a guanidine hydrochloride stable structure which is inaccessible to tyrosinase.

While these studies suggest that mussel glue protein possesses an open unordered conformation in solution, once adsorbed, the conformation need not remain unordered. An open, extended conformation in solution would suggest that all the *o*-diphenolic groups have equal probability of forming stable complexes with suitable surface sites.

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