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The Phosphoprotein of the Dentin Matrix*

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ABSTRACT: Purified, decalcified bovine dentin matrix contains a small amount of bound phosphorus but otherwise appears to have a composition typical of mammalian collagens. The bound phosphorus cannot be removed by nonhydrolytic or other nondegradative extractions. Prolonged oxidative degradation of the matrix with sodium metaperiodate resulted in the solubilization of about 30% of the matrix and this portion contained more than 75% of the phosphorus. Fractionation of the solubilized dentin led to the isolation of a rapidly moving, electrophoretically homogeneous anionic component (designated F component) at pH 5.3. Equilibrium ultracentrifuge studies showed the F component to be homogeneous with respect to molecular weight and to have a molecular weight of 38,000 ± 3000. Analyses showed F to be a protein, containing unusually large amounts of serine and aspartic acid plus smaller amounts of proline, hydroxyproline, and one residue of hydroxylysine per molecule. F contained 5.9% P by weight of 34 phosphate groups/molecule. Since hydroxylysine is destroyed under the conditions of periodate degradation unless either the hydroxyl or ε-amino group is blocked, and since the F component contained almost all of the hydroxylysine of the dentin which survived the periodate degradation it was concluded that the F component phosphoprotein is attached to the collagen matrix *via* the hydroxylysine side chain. The phosphoprotein comprises less than 2% of the total matrix on a weight basis or less than one molecule per four molecules of collagen. This highly anionic phosphoprotein, bound to the collagen matrix, may provide the sites for the epitactic nucleation of mineralization of the matrix.

The collagen of the dentin matrix has several characteristics which distinguish it from the more generally studied soft tissue collagens. The most distinctive properties of dentin matrix collagen are those of extreme resistance to dimensional change and an equally marked resistance to solubilization by all except degradative treatments. There is also, in contrast to bone matrix, a much lower rate of turnover. Glimcher and Katz (1965) have attributed these properties to an exceptionally strong interaction between neighboring molecules, forced upon the system when mineralization depletes the water content of the tissue. The peculiar matrix organization and fibrillar weave, clearly seen at the electron microscopic level (A. Veis and O. O. Mussell, unpublished results), may contribute to the structural stability. Veis and Schlueter (1964), however, proposed that the limited solubility might be the result of an especially high degree of intermolecular covalent cross-linking, in which an extra set of polymerizing bonds were superimposed on the structure in addition to the normal soft-tissue polymerization system (Schlueter and Veis, 1964). Although analytical data on the composition showed dentin collagen to be very similar to purified soft tissue collagen in terms of carbohydrate and amino acid components (Eastoe, 1963; Piez, 1961; Veis and Schlueter, 1964) one distinct difference was the presence of covalently bound phosphate in the completely demineralized dentin (Veis and Schlueter, 1964; McKernan and Dailly, 1966). While the presence of the phosphate might be an unimportant artifact, as suggested by Glimcher and Krane (1964), compact bone matrix collagen, also a highly mineralized tissue in which the matrix collagen is exposed to relatively high phosphate concentrations, contains much less covalently bound phosphate (McKernan and Dailly, 1966), and phosphate appears in only trace amounts in soft-tissue collagens (Glimcher *et al.*, 1964). Thus, it appeared to us reasonable to suppose that the bound phosphate played some definite role in the dentin matrix, in par-

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ticular, in structure stabilization or the nucleation of mineralization. We, therefore, undertook this study of the location of the covalently bound phosphate of the dentin matrix.

Periodate oxidation of the dentin matrix (Schlueter and Veis, 1964) results in the partial solubilization of the matrix collagen. One nondialyzable fraction of the solubilized protein, representing only ~6% of the total dentin nitrogen, was shown to contain ~40% of the total phosphorus of the system. Thermal hydrolytic solubilization of the dentin failed to yield any fractions particularly enriched in phosphate. We have, therefore, directed our attention to the isolation and characterization of the phosphorus-containing fragment resulting from the periodate degradation of purified bovine dentin collagen.

Experimental Section

Purified Dentin Collagen. The procedure of Veis and Schlueter (1964) was used. In brief, unerupted teeth were removed from fresh cattle jaws. The pulp and its processes were easily extracted. The adhering soft tissues and cementum were removed by scraping. Following several washes in 15% NaCl the teeth were rinsed, then broken into small pieces, and extracted repeatedly at 4° with 0.5 M EDTA adjusted to pH 7.4 with NaOH. The teeth were shaken vigorously during the extraction. The brittle enamel layer flakes off of the softer and more flexible dentin and the soluble enamel proteins are dissolved, followed by the soluble components of the dentin (Zamoscianyk and Veis, 1966). Extraction was continued until the calcium content was reduced below the limit of detection. The soft, white pieces of dentin collagen were washed with distilled water to remove all EDTA, then lyophilized for storage.

Periodate Degradation and Isolation of the Phosphate-Rich Fraction. The procedure of Grassmann and Kühn (1955) was used with slight modification (Schlueter and Veis, 1964). A 0.025 M solution of sodium metaperiodate, adjusted to pH 7.75 with an NaOH–bicarbonate buffer solution, was shaken with the dentin collagen at 40° for 28–30 hr. Pyrex "low actinic" reaction vessels were used to minimize periodate loss. A ratio of 100 ml of periodate solution/g of dentin collagen was used. At the conclusion of the extraction the pH had risen to ~8.3.

The reaction mixture was filtered and the filtrate was dialyzed against 0.01 M $NaHCO_3$ to remove excess periodate and low molecular weight degradation products. The retentate was acidified with HCl and dialyzed against 0.01 N HCl. At this acidity a precipitate formed and this was removed by centrifugation at 13,500 rpm at 5° for 1 hr in a Servall SS-1 centrifuge. The precipitate was redissolved in dilute sodium hydroxide and then dialyzed exhaustively against distilled water and, finally, was lyophilized for storage. This dried product contained the major part of the phosphorus of the system.

Chemical Analyses. Phosphorus was determined by the Gomori (1942) method. Samples were digested in 5 ml of 10 N H_2SO_4, with 2 N HNO_3 added dropwise as required. The clear digests were diluted to 10 ml with water and aliquots were taken for colorimetric measurement. After addition of acid molybdate, and before the addition of Elon, the pH of each solution was checked to be sure it was in the range for optimum color development (pH 0.5–1.5).

Amino acid analyses were made using a Spinco Model 120 analyzer run at 30–50°. Samples were hydrolyzed in 6 N HCl at 107° in sealed, evacuated tubes for 16–18 hr. All results reported are averages of at least three analyses representing entirely separate periodate digestions, fractionation, isolation, and hydrolysis steps.

Electrophoretic Analysis and Preparative Electrophoretic Isolation. The electrophoretic mobilities and homogeneity of the acid precipitate and the isolated components were determined in a Spinco Model H electrophoresis apparatus at pH 5.3 using a 0.1 M sodium acetate buffer solution. The acid precipitate contained two components with widely different mobilities. Our interest centered principally upon the more rapidly moving component. Since this could be separated very sharply from the slower moving component, direct isolation could be accomplished with relative ease. For preparative work, a 2% solution of the acid precipitate was prepared in 0.1 M sodium acetate adjusted to pH 7.2 with NaOH and dialyzed against 150 times its volume of 0.1 M sodium acetate for at least 18 hr. The solution was transferred to a standard macroelectrophoresis cell and electrophoresis was allowed to proceed until the fastest ascending boundary reached the upper limit at which it could be viewed in the schlieren optical system. The current was turned off and the upper cell assemblies were removed. The fast-moving component was easily removed by means of a long-needled syringe from the ascending channel. The slow-moving component was similarly removed from the descending channel.

Ultracentrifugal Analyses. Sedimentation coefficients were determined in the usual manner in a Spinco Model E ultracentrifuge, at 59,780 rpm, 20°, and in the pH 5.3, 0.1 M sodium acetate buffer. Weight-average molecular weights were determined in multichannel cells by the short-column sedimentation equilibrium method of Yphantis (1960). The molecular weight and homogeneity were also determined by the high-speed equilibrium method of Yphantis (1964), involving sedimentation of all material away from the meniscus. Interference optics were used and the treatment of the data from these runs followed the recommendations of Yphantis (1964). Plates were measured with a Gaertner microcomparator.

Results

The periodate degradation and solubilization reactions checked very well with the previously established results (Schlueter and Veis, 1964), and led to the distribution of materials shown in Table I. There is a clear-cut accumulation of more than 75% of the total

TABLE I: The Sodium Metaperiodate Solubilization of Decalcified Dentin Matrix.[a]

Material	% of Total Matrix Recovd (by wt)	mg of P/g	% System P
Purified dentin		3.03	100
Residual insoluble matrix	69.3	0.63	14.4
Total periodate[b] soluble	~30		
Acid-precipitate fraction	6.4	16.11	33.1
Acid soluble, non-dialyzable	14.4	8.97	42.6
Lost on dialysis	9.2		

[a] Reaction at 38°, 28 hr. [b] Not determined directly.

system phosphorus in the nondialyzable portion of the periodate-solubilized fraction. The acid-precipitate fraction, which accounts for only 6% of the total system nitrogen, contains almost 34% of the system phosphorus, an 80-fold enrichment.

Electrophoretic analyses of the acid-precipitate fraction (Figure 1a) showed that only two components were present. The fast component, at pH 5.3 in sodium acetate buffer, has a mobility, μ, of -14×10^{-5} cm²/v sec. The relative component peak areas indicated that the fast component appeared to comprise about 5% of the acid precipitate.

Preliminary attempts at chromatographic fractionation showed marked losses of the fast component due to absorption on the exchange resins used. Since it was present in such small amounts (only 5% of 6% of the initial dentin or 0.3%) losses were not tolerable. Hence, we resorted to the cumbersome but direct electrophoretic preparative separation technique described in the Experimental Section. The fast (F) and slow (S) components isolated in this fashion were electrophoresed after purification, with the results show in Figure 1b,c, respectively. Both components were essentially electrophoretically homogeneous (less than 5% of S in F and *vice versa*).

Amino acid analyses of the initial insoluble dentin matrix, the total acid-precipitate fraction (undialyzed), and the isolated F and S components of the precipitate fraction are given in Table II in terms of residues of amino acid/1000 total amino acid residues. The insoluble decalcified dentin matrix has a composition typical of mammalian collagens, in which glycine, alanine, proline, and hydroxyproline together account for 66.7% of the residues of the protein. The S component is similar to the parent collagen but is atypical in that glycine, alanine, proline, and hydroxyproline make

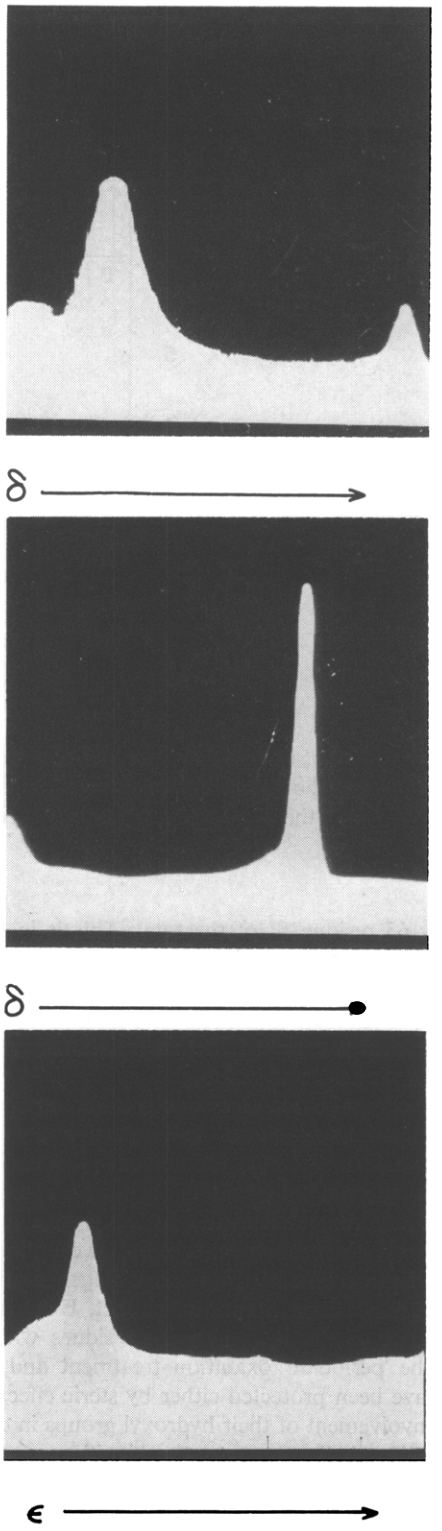

FIGURE 1: Electrophoretic patterns of the periodate-solubilized fractions of dentin collagen obtained during preparative electrophoretic isolation. All runs in 0.10 M sodium acetate (pH 7.2) at 1°. Macro-cell, Spinco Model H electrophoresis: (a) (top) ascending channel, 145 min, 20 ma; (b) (middle) ascending channel, F component, 107 min, 16 ma; and (c) (bottom) descending channel, S component, 75 min, 20 ma.

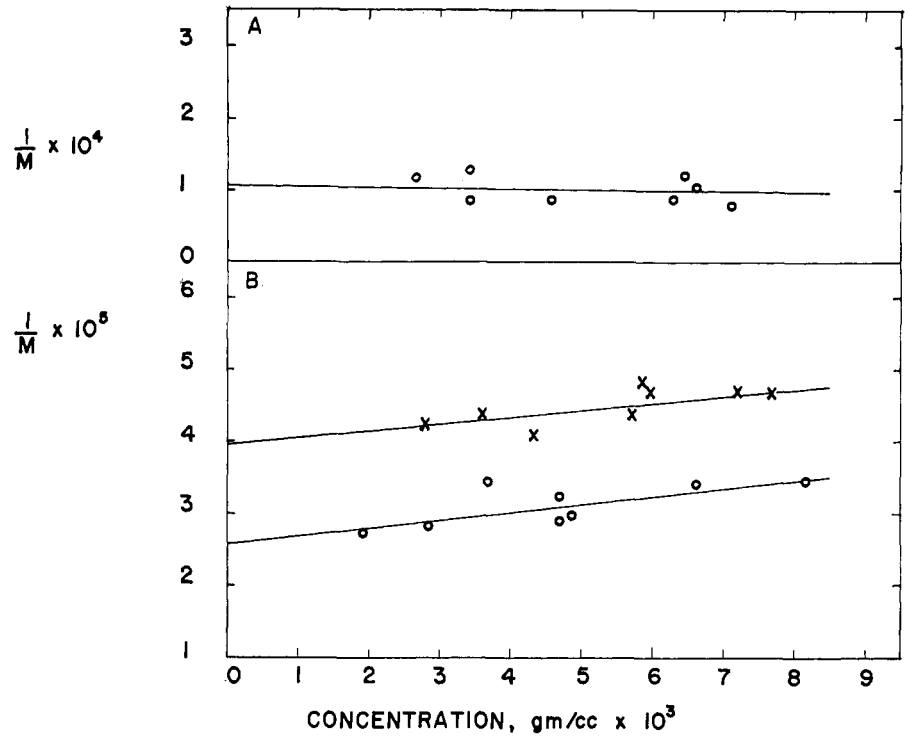

FIGURE 2: Reciprocals of the molecular weight for the various fractions solubilized by periodate. Data obtained by the midpoint method in short-column multichannel cells. (A) (upper figure) The periodate-solubilized fraction not precipitated when the periodate extract is acidified. (B) (lower figure) ●, F component; x, S component.

up only 63 residue % of the total. This is in spite of the fact that tyrosine is absent and hydroxylysine and methionine are low because they are destroyed during the prolonged periodate oxidation. The amino acids markedly enriched in this fraction are aspartic and glutamic acids and serine.

The F component has a distinctly different, essentially noncollagen, composition. It is particularly rich in aspartic acid and serine (combined ~75 residue %) and has only modest amounts of glycine, alanine, and proline. Hydroxyproline and hydroxylysine are both present as distinct constituents of F, however, indicating that F has originated from the collagen matrix and is not a noncollagen impurity. In fact, F contains the major part of the hydroxylysine residues which survived the periodate oxidation treatment and, hence, must have been protected either by steric effects or by direct involvement of their hydroxyl groups in covalent bonds. We shall return to the significance of these results after the data on the homogeneity of F and S are discussed.

Phosphorus analyses showed F to contain 59.3 mg of P/g and S, 8.5 mg of P/g. On a phosphate moiety basis these values represent 94 and 8.9 phosphate groups/1000 amino acid residues in F and S, respectively (Table II).

Examination of the amino acid content and phosphorus data for the total acid-precipitate fraction and for F and S shows that it is not possible to make a complete material balance for either every amino acid or the phosphorus. Some constituents are lost on dialyses and on isolation of the F and S components. For those constituents for which no losses are evident, the analytical data are consistent with a 5–7% weight content of F in the acid precipitate. The phosphoserine contents noted in Table II should be regarded as minimum values. Phosphoserine is acid labile and most is lost during protein hydrolysis. While it is not certain, of course, that all the phosphate is on serine, there is more than enough serine to accommodate all of the phosphate as monophosphate esters.

Weight-average molecular weights for F and S were determined by the short-column equilibrium method of Yphantis (1960) as a function of the protein concentration. Figure 2 shows plots of the concentration dependence of the apparent M_W for each component. The lines represent least-square fits for these data and yield zero concentration extrapolated values of $M_{W,F}$ = 38,500 and $M_{W,S}$ = 25,200. Direct determination of the partial specific volumes, $\bar{v}$, were not made due to the small quantities of protein available. The value of $\bar{v}_S$ was taken as 0.709, a value appropriate for collagen and gelatin (Veis and Drake, 1963; Heaps *et al.*, 1966). The unusual composition of F precludes the use of $\bar{v}_S$; therefore, $\bar{v}_F$ was computed from the composition data and the residue $\bar{v}$ values of Edsall and Cohn (1943) and found to be 0.656. Figure 2A shows the results of similar short-column runs for the soluble fraction *not* precipitated when the pH is adjusted with the HCl. Since this fraction has an average com-

TABLE II: Amino Acid Compositions of Dentin Collagen and Fast and Slow Components of the Acid Precipitate from the Periodate Solubilization.[a]

Amino Acid	Components			
	Dentin	Acid Ppt	Slow	Fast[b]
Aspartic acid	49.9	80.4	72.1	365
Serine	38.2	59.4	53.2	375
Phosphoserine	0.5	4.3	1.5	14.5
Threonine	17.0	15.8	16.8	0.0
Proline	117.8	90.5	98.6	20.3
Hydroxyproline	98.7	62.8	95.8	17.2
Glycine	325.8	328	323	72.3
Alanine	124.2	119	113	21.4
Valine	20.7	25.1	21.5	6.9
Leucine	24.7	23.4	22.7	8.3
Isoleucine	11.1	8.9	9.6	5.0
Phenylalanine	11.9	11.2	11.2	2.9
Tyrosine	4.0	0.0	0.0	0.0
Methionine	5.9	2.8	3.0	0.5
Glutamic acid	70.9	83.5	86.4	27.1
Histidine	5.2	6.2	2.1	2.3
Lysine	18.9	24.4	22.4	45.7
Hydroxylysine	9.2	0.9	1.1	2.8
Arginine	46.9	44.2	44.8	9.9
(Ammonia)	(37)		(71)	(198)
(Phosphate)	(5.8)		(8.9)	(94)

[a] In residues/1000 residues. [b] Average of four analyses of four separate preparations ±5%.

position near that of collagen $\bar{v}_{sol}$ was taken as 0.709. The weight-average molecular weight of this fraction is on the order of 10,000.

The electrophoretic mobility data and the direct preparative electrophoretic technique demonstrate, and indeed require, that F be electrophoretically homogeneous. The molecular weight heterogeneity was tested by the high-speed equilibrium ultracentrifugation technique of Yphantis (1964) at several concentrations. Figure 3 shows the basic data plot of the log of the fringe displacement *vs.* microcomparator X coordinate for a 0.1% solution of F, for fringe displacements of 50 μ and greater. The nonlinearity evident in this plot indicates either a marked concentration dependence or that F is heterogeneous. Point-average values of the molecular weight function, $\sigma(r)$, from the slopes of adjacent data point regions in the plot of Figure 3 are shown in Figure 4, plotted as $\sigma(r)$ *vs.* the concentration (fringe displacement units). The extrapolated value of $\sigma(r)$ leads to a zero concentration weight-average molecular weight of 37,000 ± 3000. This molecular weight should represent the "weight" of the lowest molecular weight component in the solution. Since it agrees so well with the extrapolated molecular weight from the short-column low-speed equilibrium centrifuge

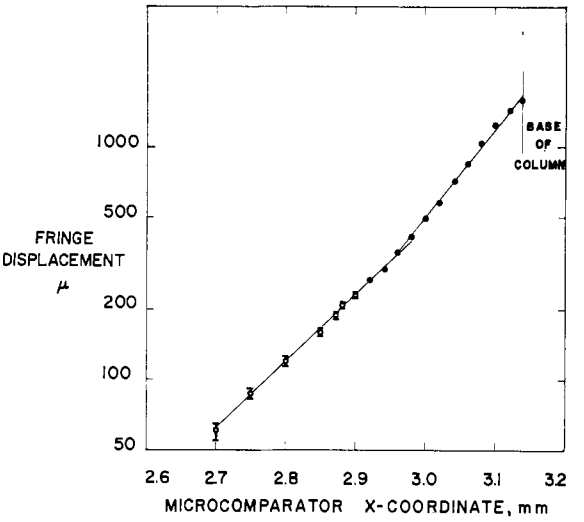

FIGURE 3: Fringe displacement *vs.* microcomparator X coordinate for the F component, 0.1% solution, high-speed equilibrium centrifugation run, 25,980 rpm.

runs (Figure 2) where the average is taken over the entire solution it seems reasonable to conclude that F is very nearly homogeneous and that the nonlinearity of the log fringe displacement X coordinate plot is the result of concentration dependence terms. Plots of $\sigma(r)$ *vs.* $(b^2 - r^2)$ (where r is the position in the cell at which σ is computed and b is the radial distance of the base of the cell from the center of rotation) for solutions of different initial protein concentration form a set of independent lines, further indicating a concentration dependence rather than heterogeneity (Yphantis, 1964). A marked concentration dependence is not surprising for a system of high charge density macromolecules.

Discussion

The data presented in the previous section demonstrate unequivocally that at least one moiety, the component with the higher electrophoretic mobility, rich in serine, aspartic acid, and phosphate residues, is present in demineralized and exhaustively EDTA-extracted dentin. Of the several questions which can be raised with regard to the origin and significance of this substance, the most crucial is that of the nature of the association between this moiety and the collagen matrix. Is it a part of the cellular debris trapped within the tightly woven collagen matrix or is it an integral part of the matrix?

Several lines of evidence suggest that the F component was an integral part of the matrix structure. Although the dentin matrix resists swelling throughout the pH range from 2 to 11 (Veis and Schlueter, 1964) anionic sialoglycoproteins of high molecular weight can be extracted from the unswollen matrix at pH 7.4 by EDTA (Zamoscianyk and Veis, 1966) and similar glycoproteins

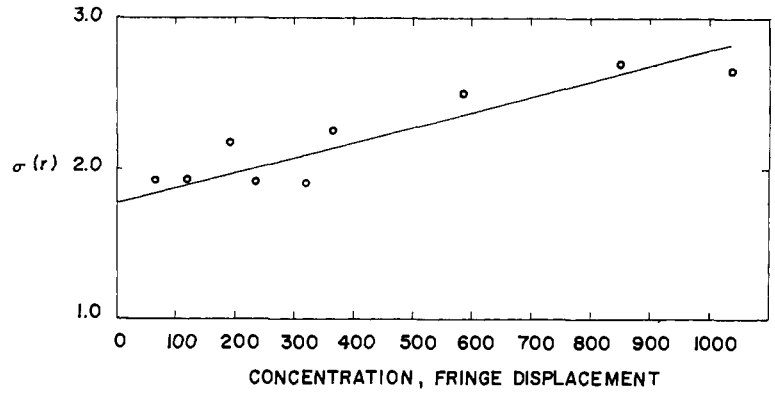

FIGURE 4: Plot of the molecular weight function, $\sigma(r)$, *vs.* concentration for the F component from the basic data shown in Figure 3. The extrapolated value yields the zero concentration molecular weight.

have been extracted from nonswollen compact bone (Herring, 1964). Since the F component has similar charge characteristics it would seem likely to suppose that it should be extractable under similar conditions if in a nonbonded state. Extractions with acids, with denaturants such as 8.0 M urea and 2.0 M KCNS, with concentrated neutral salts, or even with basic solvents such as liquid ammonia, all failed to remove the phosphate-containing moieties from dentin (Veis and Schlueter, 1964) indicating that these moieties must be intimately associated with the matrix and not mechanically trapped. The decalcified matrix is, in fact, a highly porous structure with many channels permitting rapid diffusion of reagents into the interior of the fibrils even when the dentin is not highly divided.

A comparison of the thermal hydrolytic solubilization and the periodic acid oxidative solubilization of the dentin also suggests that the F component was firmly associated with the matrix. When identical relative amounts of the dentin, *e.g.*, 50%, are brought into solution by the two routes and fractionated and analyzed in the same way, no fraction comparable to the F component was obtained by the thermal hydrolytic procedure (Schlueter and Veis, 1964).

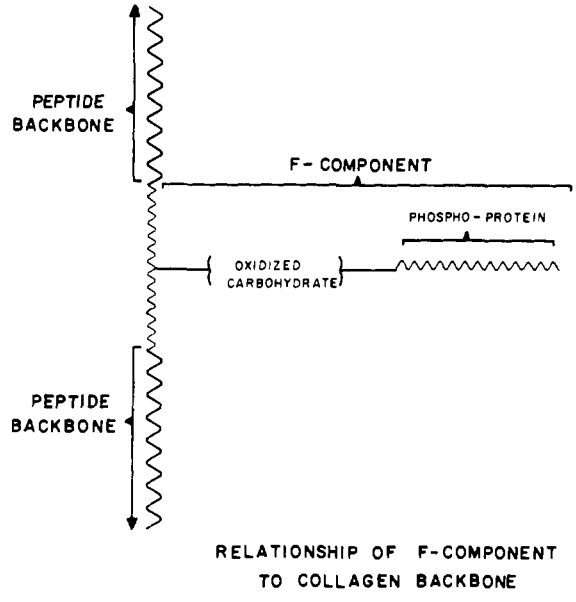

FIGURE 5: A schematic view of the proposed relationship of the F component to the collagen matrix.

The most compelling arguments in favor of the conclusion that the F component was an integral part of the matrix lie in the homogeneity and composition of the F component itself. The F component appears to be homogeneous by both electrophoretic and ultracentrifugal techniques. As shown in Table II, this homogeneous component contains substantial amounts of both proline and hydroxyproline, and the proline: hydroxyproline ratio is the same as in the original undegraded dentin matrix. Further, the F component contains almost all of the hydroxylysine which survived the periodate oxidation. Hydroxylysine is an amino acid occurring almost exclusively in collagen.

The side chain of hydroxylysine is readily attacked by periodate under the conditions utilized, yielding both ammonia and formaldehyde (Hörmann and Fries, 1958). To be susceptible to oxidation by periodate both the vicinal hydroxyl and amino group must be free or nonbonded. Thus, the failure of periodate to destroy the hydroxylysine of the F component is strong evidence indicating that either the amino or hydroxyl group is involved in a covalent bond.

Butler and Cunningham (1966) have found that there is also a small amount of hydroxylysine in citrate-soluble guinea pig skin collagen which resists oxidation by periodate. By a series of tryptic and collagenase digestions, followed by extensive chromatographic isolation procedures, they demonstrated that the hydroxylysine-containing peptide also contained a disaccharide of glucose and galactose and that this was linked *O*-glycosidically to the hydroxyl group of the hydroxylysine. A similar situation may prevail in the F component. Schlueter and Veis (1964) found an anthrone-reactive component similar to a hexuronic acid

to be present in the dentin solubilized after reaction with periodate for 30 hr. They suggested that some of the hexose might be involved in 1–3 or 1–4 linkage so that it, too, would not have the pyranose ring structure degraded during the periodate oxidation.

The above considerations lead us to propose that the F component, a fragment produced *via* a degradation of the native structure, is composed, as illustrated in Figure 5, of three sections. The first section, containing proline, hydroxyproline, and hydroxylysine, among other amino acids, is a part of the peptide backbone of the dentin collagen. The second section is an oligosaccharide, possibly oxidized at a C-6 due to the prolonged oxidative treatment, linked so that it retains the pyranose form. Rodén and Smith (1966) have recently demonstrated the presence of a 1–3-linked neutral trisaccharide in the chondroitin sulfate–protein linkage region. The final section, linked to the oligosaccharide, is a typical phosphoprotein or peptide rich in serine, phosphoserine, and aspartic acid.

The weight-average molecular weight of the F component is surprisingly high, ~37,000, but is quite consistent with both the limited backbone degradation occasioned by the periodate and the composition data of Table II. Assuming one hydroxylysine residue per degradation fragment, the composition of the F component is as indicated in Table III, and yields a calculated minimum molecular weight of 35,000.

In the native bovine dentin used there are 9.5 residues of hydroxylysine/1000 total amino acid residues, or ~30 residues/collagen monomer unit. Only about 1% of this hydroxylysine resists degradation by periodate, equivalent to only one protected hydroxylysine side chain per three to four collagen monomer units. This corresponds to a maximum of only ~2% F component by weight in the decalcified, EDTA-extracted matrix. A much smaller amount than this was actually isolated. The very small amount of the F component present explains our inability to detect significant differences between the over-all composition of the purified, but undegraded, dentin matrix and the composition expected for a typical pure collagen.

TABLE III: Calculated Molecular Composition of the F Component on the Basis of 1 Mole of Hydroxylysine/Molecule.

Amino Acid	Residues/Molecule
Aspartic acid	130
Serine	134
Phosphoserine	5
Proline	7
Hydroxyproline	6
Glycine	26
Alanine	8
Valine	2
Leucine	3
Isoleucine	2
Phenylalanine	1
Glutamic acid	10
Histidine	1
Lysine	16
Hydroxylysine	1
Arginine	4
(NH_3)	(70)
(HPO_3)	(34)[a]

[a] Includes the five residues of phosphate in the serine phosphate.

In a number of papers, Schmitt and his colleagues (Schmitt, 1964; Rubin *et al.*, 1963, 1965; Drake *et al.*, 1966; Davison and Drake, 1966) have demonstrated that acid-soluble collagens from soft tissues contain peptide appendages that can be cleaved from the main body of the collagen molecule by proteases. It is not clear whether these peptides originate from the ends of the molecule or are distributed along the trihelical body; however, the peptides obtained have one common characteristic. They are all of low molecular weight. Schmitt (1964) has argued that these protease-susceptible peptides represent sites at which cross-linking takes place, and which otherwise modify the tissue-specific interactions between collagen monomer units. The phosphoprotein moiety of the F component is quite different in nature. It represents a high molecular weight component present in only small concentration in the matrix. It may, however, play an equally important role in regulating the properties of the matrix. Considering that the phosphoprotein moiety appears to be attached to the matrix collagen at only one point per collagen monomer it is unlikely that this component takes part in the cross-linking of the collagen. It is more likely that the phosphate rich, highly anionic phosphoprotein moiety provides the sites for the nucleation of the epitactic mineralization of the dentin.

Hodge and Petruska (1963) have demonstrated the presence of a systematic set of "holes" or spaces which run through native collagen fibrils at an axial spacing of ~2800 A along any particular microfibril. Glimcher *et al.* (1965) have suggested that mineralization is initiated within these "hole" regions. Since the collagen monomer rods are in a close-packed array, one phosphoprotein moiety per "hole" would correspond to one per four to six collagen monomer molecules. Localization of the phosphoprotein moiety to the "hole" regions would also place it at or near an end region of the collagen molecule. In this case, only a single peptide backbone cleavage would be required to release the phosphoprotein-containing F component in soluble form from the matrix.

In summary, we have demonstrated that a high molecular weight phosphoprotein is covalently attached to the collagen of the matrix. It is probable that this attachment is through the side chain of a hydroxylysine residue and may involve an oligosaccharide. The phosphoprotein is present in an amount corresponding to less than 2% of the total protein of the dentin matrix, or in a ratio of one molecule of phosphoprotein to four to six molecules of collagen. The highly anionic

phosphoprotein could serve as the site, within the "hole" regions of native dentin collagen, for the epitactic nucleation of mineralization of the matrix.

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