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IDENTIFICATION OF SERINE PHOSPHATE IN A PROTEIN FRACTION OCCLUDED IN THE MINERALIZED MATRIX OF CALCIFIED HUMAN AORTA

F. W. Keeley
Research Institute
The Hospital for Sick Children
Toronto, Canada
M5G 1X8

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SUMMARY

We have previously described an acidic, organic phosphorous-containing protein fraction prepared from calcified regions of atherosclerotic human aorta. This communication demonstrates the presence of serine phosphate in this protein fraction.

INTRODUCTION

It is now generally accepted that matrix factors, at least some of which are protein in character, play an important role in the mineralization process in normally calcifying tissues, bones and teeth. Several of these matrix proteins have been shown to contain organic phosphorous in the form of serine phosphate, sometimes in very high proportions (1-5), and it is thought that this phosphorylated amino acid may provide binding sites for calcium (6,7).

Compared to these physiologically mineralizing tissues, the mechanism for the initiation of focal, pathological calcifications, such as those found in atherosclerotic aortic tissue, is not well understood. We have been investigating the possibility that matrix proteins are also involved in mineralization at these sites. We have previously described an acidic, cholesterol-associated protein fraction isolated from calcified plaques of atherosclerotic human aorta (8). This protein fraction also contained organic phosphorous. In the present communication we demonstrate that at least some of this organic phosphorous can be accounted for by the presence of serine phosphate in the protein fraction.

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METHODS

Methods for the detection of serine phosphate are complicated by the acid lability of the substance and the fact that, on amino acid analysis in most commonly used systems, serine phosphate co-elutes with cysteic acid, an oxidation product which may arise under acid hydrolysis conditions from cysteine in the protein.

In this study, cysteic acid and serine phosphate were separated on a Durrum D-500 amino acid analyzer by a modification of the method of Glimcher et al (9) using a buffer of pH 1.4 prepared by acidification with HCl of a 0.2M sodium citrate buffer, pH 3:25, containing 0.05% thiodiglycol and 0.1% phenol (Pierce Chemical Co).

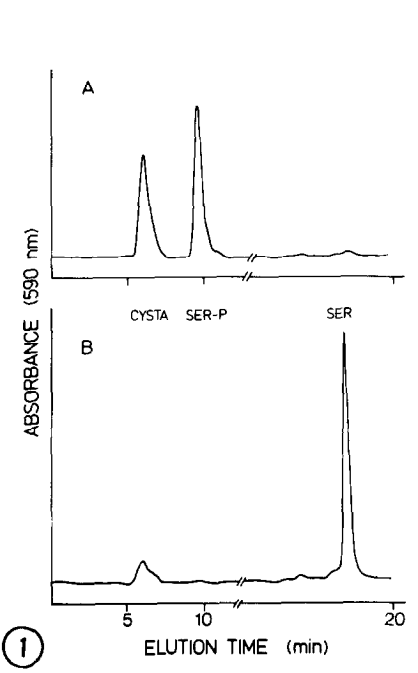
The protein fraction used was peak II from DEAE Cellulose chromatography, a component of calcified regions of atherosclerotic human aorta prepared and characterized as described elsewhere (8). Weighed portions of the protein fraction were hydrolyzed in 4M HCl for 5 hours at 110° in vacuum and reconstituted in 0.01M HCl for amino acid analysis.

Recovery of serine phosphate in the hydrolysis procedure was determined using α-casein (Sigma Chemical Co) hydrolyzed under the same conditions. The phosphorous content of α-casein was determined by the method of Bartlett (10). Assuming that all phosphorous in α-casein is present as serine phosphate, the serine phosphate content of the protein fraction can be corrected using the value for the recovery of serine phosphate from α-casein.

For confirmation of the identity of the peak on amino acid analysis as serine phosphate, a sample of the protein fraction was hydrolyzed in 4M HCl for 5 hours at 110° under vacuum, reconstituted in 0.01M HCl, and chromatographed on a 0.5 cm x 5 cm column of Dowex 50x8 (H^+ form), eluting with 0.01M HCl. Under these conditions, cysteic acid and serine phosphate are not retarded by the ion exchange resin while all other amino acids are retained. The first 10 ml of eluant was collected, concentrated, and analyzed for serine and serine phosphate. An aliquot of this concentrated eluant was re-hydrolyzed in 5.7M HCl for 16 hours at 110° under vacuum and similarly analyzed.

RESULTS AND DISCUSSION

The separation of standard serine phosphate and cysteic acid on amino acid analysis using the pH 1.4 buffer system is seen in Figure 1, together with a similar analysis of the protein fraction from calcified regions of atherosclerotic human aorta. These data show that there is a peak in the protein fraction which corresponds to the elution position of standard serine phosphate. The identity of this peak is further confirmed by isolation of the putative serine phosphate (together with cysteic acid) from a 4M HCl hydrolysis on Dowex 50x8 and demonstration by amino acid analysis before and after further



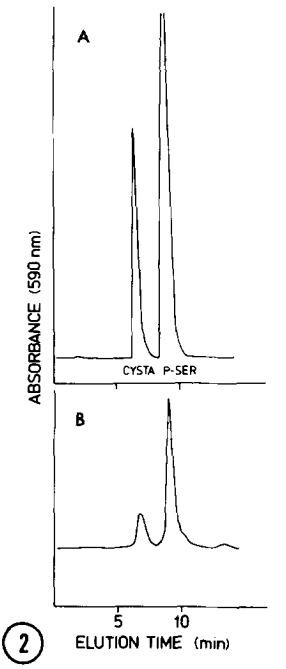


Figure 1: Amino acid analysis using 0.2M sodium citrate buffer, pH 1.4.
A: authentic cysteic acid and serine phosphate.
B: partial acid hydrolysis of protein fraction prepared from calcified aorta.

Figure 2: Amino acid analysis of Dowex 50x8 eluant before (A) and after (B) complete acid hydrolysis. The elution positions of cysteic acid, serine phosphate and serine are indicated.

hydrolysis in 5.7M HCl that this peak is converted to serine on complete hydrolysis (Figure 2). No evidence for the presence of the threonine phosphate, which is also resolved by the amino acid analysis system, was seen.

Quantitation of the serine phosphate peak from Figure 1B, correcting for the recovery of serine phosphate from α-casein analyzed under the same conditions, indicated that 6 residues per thousand of the total of 71 residues per thousand of serine present in the protein fraction after complete hydrolysis (8) are phosphorylated. Since the protein fraction is not homogeneous and not all components may contain serine phosphate, it is therefore possible that the fraction includes a protein or proteins

whose serine phosphate content is higher than this value calculated for the mixture.

Proteins containing serine phosphate, sometimes in large proportions have been isolated from the mineralizing sites of bones and teeth, (1-5) and it has been suggested that this phosphorylated amino acid serves as a calcium binding site for the function of these proteins in the initiation and control of calcification (6-7). The identification of serine phosphate in proteins occluded in the mineralized matrix of a pathologically calcified tissue such as atherosclerotic human aorta raises the possibility that similar matrix factors may be involved in the mechanism of pathological calcification.

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