CALCIUM ION EFFECTS A NOTABLE CHANGE IN ELASTIN CONFORMATION BY INTERACTING AT NEUTRAL SITES

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SUMMARY: Solubilized α-elastin dissolved in trifluoroethanol with 2.5% water and in trifluoroethanol with 4% trifluoroacetic acid and 2.5% water undergoes a large conformational change on addition of $CaCl_2$ which is not observed on addition of NaCl, HCl or Na acetate. In the presence of trifluoroacetic acid all groups on the elastin protein are either positively charged or neutral. The ability of calcium ion to effect a large conformational change in the presence of a strong organic acid demonstrates that it is interacting at neutral sites and provides experimental evidence for a recently proposed mechanism of calcification in which the driving force for calcification is the affinity of calcium ion for neutral sites.

INTRODUCTION: It has been demonstrated in the aorta that the component with greatest affinity for calcium ion is the elastic fiber (Yu 1967). In his description of the process of atherosclerotic plaque formation, Moon (1967) describes an initial phase of "rupture, fragmentation and fraying of the internal elastic membrane." In the advanced degenerative phase Moon states that "the internal elastic membranes may be completely absent at the bases of the large arteriosclerotic plaques," and that "calcium deposits become visible in the area formerly occupied by the internal elastic membrane." The question of fundamental interest is the nature of the calcium ion binding site which would initiate calcification and plaque formation. Sulfhydryl groups have been implicated in initiating calcification (Schiffmann et al. 1966) as have carboxylate anions in combination with threonine hydroxyls (Hall 1955) or in combination with amine groups (Molinari-Tosatti et al. 1968). Yet these amino acids and groups are

at extremely low concentration or are absent in the carefully determined amino acid composition of elastin (Petruska and Sandberg 1968 and Franzblau and Lent 1969).

Recently it has been proposed that elastin binds calcium ion at neutral sites utilizing the peptide oxygens as coordinating groups (Urry in press). This is based on the affinity of neutral polypeptide antibiotics for alkali metal ions in which the coordinating groups are the acyl oxygens of peptide and ester moieties (Pinkerton et al 1969, Ovchinnikov et al. 1969 and Ohnishi and Urry 1970). Affinity of calcium ion for neutral sites provides the basis for a charge neutralization mechanism for calcification. Briefly the mechanism is as follows: The affinity of calcium ion for neutral sites on elastin results in a space charge saturation of the elastin matrix. The charge saturated matrix then attracts counter ions, such as phosphate and carbonate, which neutralize specific sites and allow adjacent sites to bind calcium, etc., until nucleation has occurred. The present report demonstrates the affinity of calcium ion for neutral sites on solubilized elastin.

EXPERIMENTAL: Solubilized elastin was prepared by the method of Partridge et al. (1955) from bovine ligamentum nuchae. This treatment results in two protein components, α-elastin of 70,000 mw and a β-elastin of 5,500 mw. The two components were separated on Sephadex G-100. The trifluoroethanol (TFE) was obtained from Halocarbon Products, Hackensack, New Jersey, and purified according to Krivacic and Urry (1970), and the trifluoroacetic acid (TFA) was obtained from Eastman Organic, Rochester, New York, and purified by distillation over P_2O_5.

The circular dichroism spectra were obtained on a Cary Model 60 spectropolarimeter with a Model 6001 circular dichroism accessory. The specified mgms of salts were added to one milliliter of

protein solution leaving an elastin concentration of 1.75 mgs/ml. Path length used was 0.1 mm. Weight based on concentrations was verified by determination of total nitrogen on a Coleman Model 29 nitrogen analyzer.

RESULTS AND DISCUSSION: Fig. 1 contains the circular dichroism data on α-elastin in TFE with 2.5% water. Comparing figures 1-a and 1-b it is seen that calcium ion is responsible for the large

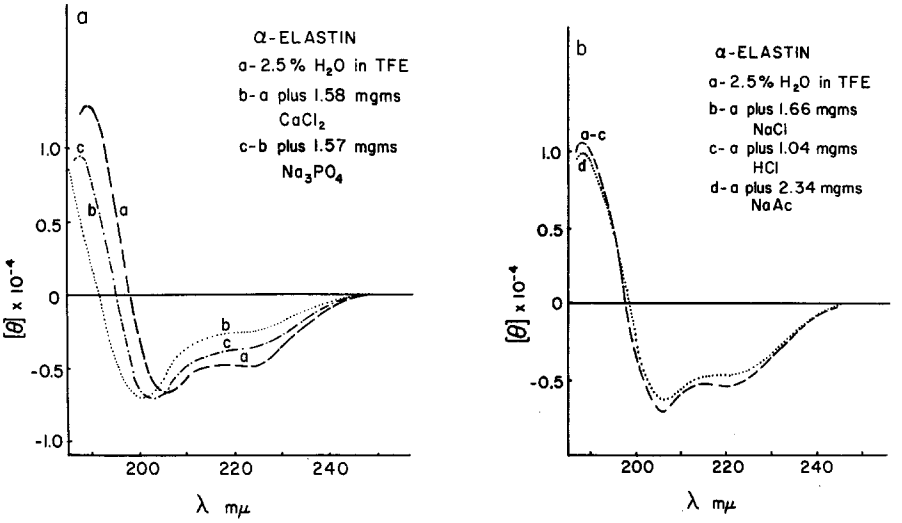

Fig. 1. Circular dichroism (CD) patterns of α-elastin in trifluoroethanol (TFE) containing 2.5% water and various salts.
1-a. Addition of $CaCl_2$ causes a marked change in the CD pattern (curve b) which is partially reversed by subsequent addition of Na_3PO_4 (curve c). Na_2CO_3 also causes partial reversal (curve not included).
1-b. Addition of NaCl, HCl or NaAc has little effect.

change in the circular dichroism pattern. Fig. 2 demonstrates that addition of trifluoroacetic acid does not alter the calcium ion specific response. Similar results were obtained with β-elastin.

It is well appreciated by those who have used strong organic acids in organic solvents for solubilizing polypeptides that, in these solvent systems, all side chains are either neutral or positively charged. TFA is such a strong protonating agent that protona

tion of the peptide oxygen has been proposed. That this does not occur in TFE-TFA solutions was demonstrated by the undiminished presence of the CD band arising from peptide n-π^* transitions of helical poly-L-alanine (Quadrifoglio and Urry 1968 and Quadrifoglio and Urry 1967).

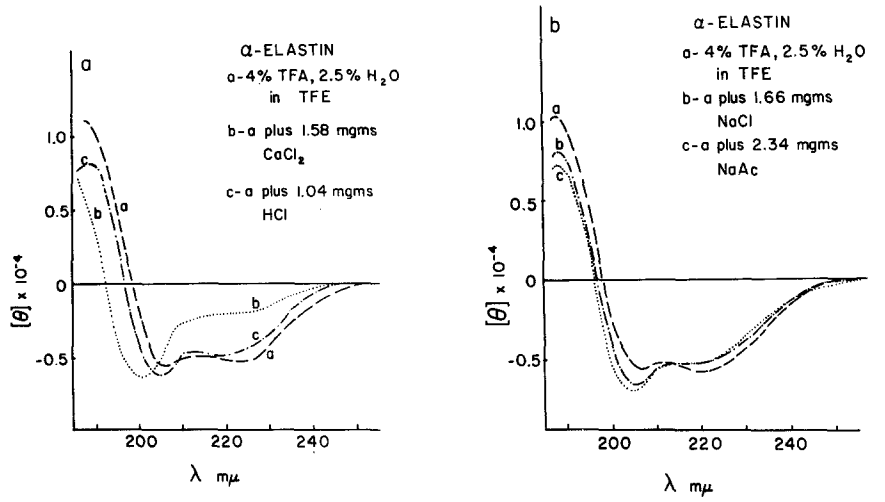

Fig. 2. Circular dichroism (CD) patterns of α-elastin in trifluoroethanol (TFE) containing 4% trifluoroacetic acid (TFA) and 2.5% water.
2-a. Addition of $CaCl_2$ again causes a marked change in the CD pattern (curve b), HCl has only a small effect (curve c). As in Fig. 1-a subsequent addition of Na_3PO_4 and Na_2CO_3 partially reverse the $CaCl_2$ effect (curve not included).
2-b. Addition of NaCl and NaAc have small effects.

Previous studies on solubilized elastin have shown that elastin, dissolved in water buffered with 0.01 M sodium acetate at pH 5 undergoes a marked conformational change on coacervation (Urry et al 1969), which is achieved by raising the temperature to 37° C. The conformational transition can be partially effected by addition of organic solvents such as ethanol (Mammi et al. 1968) or trifluoroethanol. Accordingly the CD pattern in TFE and TFE-TFA solutions resembles that of the coacervate. The effect of calcium ion is to revert the conformation toward that of the buffered aqueous solution

It is clear that the change in the CD patterns on calcium ion binding is more than loss of rotational strength due to removal of n-π* transitions on interaction with the cations. The blue shift of the negative and positive bands clearly indicates substantial conformational change.

It is seen that NaCl has little effect and that the chloride and acetate anions have little specific effect (see Figs. 1 and 2). Interestingly, addition of sodium carbonate or sodium phosphate causes substantial relaxation of structure toward the conformation in trifluoroethanol (see Fig. 1-a). A conformation more characteristic of less polar solvents is obtained even though the ionic strength of the medium is increased by addition of multivalent anions. Two possible explanations for this latter effect are extraction of the bound calcium ion by phosphate or carbonate anions and/or a more effective screening of the charge repulsion existing between the calcium ion binding sites. Accordingly the conformational change may be due to a direct change of polypeptide backbone conformation at the binding site and also due to a charge repulsion resulting in a more extended conformation. The observed changes do bear a resemblance to the pH elicited conformational transitions observed with poly-L-glutamic acid (on raising the pH from 5 to 7) and with poly-L-lysine on protonation of the side chain.

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