ISOLATION OF TWO PEPTIDES FROM ACID HYDROLYZATES OF ELASTIN (*)

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SUMMARY: Two peptides valyl-valine and leucyl-valine were isolated from 18 hour HCl hydrolyzates of bovine ligamentum nuchae elastin by a combination of gel filtration on Bio-Gel P-2 and partition chromatography on Sephadex G-25. The amounts of both peptides recovered from elastin were approximately 0.5%. The extreme resistance of these peptides to acid hydrolysis may be attributed to steric effects produced by their valine residue(s). These effects may play a role in producing the observed property of elasticity of this protein by interfering with the usual hydrogen bonding in the peptide α-helices. Such a condition may lead to a reduced rigidity, or greater extensibility of the peptide chains.

Peptide linkages between certain amino acids, in particular those containing valine in the position N-terminal to the peptide bond are very resistant to acid hydrolysis. It has been shown (1) that the synthetic peptide valyl-glycine is split at a rate of only 0.017 compared to glycyl-glycine. In view of the large number of such resistant linkages in elastin, it has become the practice when studying the composition of this protein to employ hydrolysis times as long as 72 hours (2,3). More than half a century ago the peptides alanyl-leucine (4) and leucyl-alanine (5) were isolated from sulfuric acid hydrolyzates of elastin. More recently (6) valyl-proline was isolated from an alkaline hydrolyzate of elastin. The present report describes the isolation and identification of two valine containing dipeptides, valyl-valine and leucyl-valine from 18 hour hydrochloric acid hydrolyzates of bovine ligamentum nuchae elastin. In addition to their resistance to hydrolysis, these peptides also show a greatly reduced reactivity with the ninhydrin reagent, and a low degree of polarity, giving R_f values greater than leucine on butanol-acetic acid-water paper chromatograms.

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MATERIALS AND METHODS: Five grams of bovine ligamentum nuchae elastin were hydrolyzed for 18 hours at 100° in sealed tubes with 6N HCl. The excess of acid was removed by evaporation on a steam bath, and the last traces by ion exchange on a 2.5 x 20 cm. column of Ag 50W X-2 resin. The resin was in the H + form, 200-400 mesh and was previously equilibrated with 0.2 M acetic acid. The hydrolyzate was applied to this column in 20 ml water, followed by elution with 0.2 M acetic acid until the HCl had all been washed out. Then the eluant was changed to a pH 5.0, 0.2 M triethylamine-acetate buffer, and elution continued until the very pronounced pigment zone was completely removed from the column. This pH 5.0 fraction was air-dried and subjected to gel filtration on a column of Bio-Gel P-2, 2.5 x 200 cm, using a triethylamine-acetate buffer, pH 8.4, 0.1 M as eluant. The individual tubes were monitored for their ultraviolet absorption in a Beckman DU-2 spectrophotometer, and for their reactivity with the ninhydrin reagent by the method of Rosen (7). The elution pattern so obtained is given in figure 1. The large ninhydrin positive peak IV contained the great bulk of the free amino-acids of the hydrolyzate. The two peptides

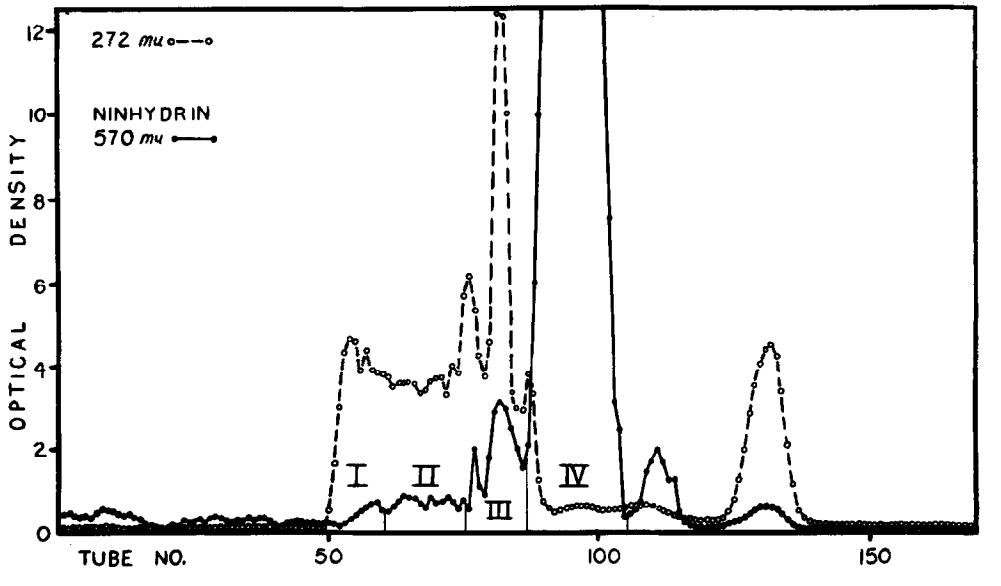

Fig. 1 Fractionation of 18 hour elastin HCl hydrolyzate on a column of Bio-Gel P-2.

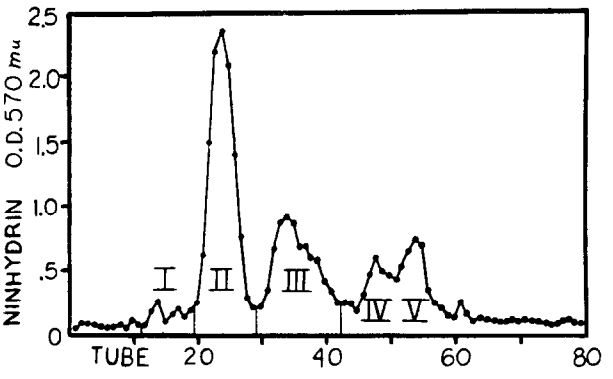

Fig. 2 Fractionation of peak III from Bio-Gel column by partition on column of Sephadex G-25, using butanol, acetic acid, water 4:1:1 solvent.

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were found in the region of the elution curve designated as III and isolated by partition chromatography of that fraction on a 1.2 x 100 cm column of Sephadex G-25 (fine beads) with N-butanol: acetic acid: water (4:1:1) as the eluant. The effluent was monitored using the ninhydrin reagent, and the resulting elution diagram is given in figure 2. Peak I contained pigment, IV was free leucine, V was free valine and peaks II and III contained the peptides. The yields of these peptides were 0.3% of the weight of the elastin for SL-2 and 0.2% for SL-1. The amino acid analysis of the peptides was done on a Technicon amino acid analyzer after hydrolysis with 6N HCl at 100°C for 144 hours. The C-terminal residue of peptide SL-2 was determined by hydrazinolysis (8)

RESULTS AND DISCUSSION: The amino acid analyses of the isolated peptides after 6 days of hydrolysis revealed SL-1 to be composed entirely of valine, while SL-2 contained equimolar amounts of leucine and valine. Figure 3 is a paper chromatogram of the isolated peptides, with a butanol-acetic acid-water 4:1:1 solvent, ascending, before and after acid hydrolysis for 18 and 144 hours. This shows the slow rate of hydrolysis, as well as the amino acids produced as a result of this treatment. Gel filtration on long columns (250 X 1.2 cm.) of Sephadex G-10 using an eluant of 1M NaCl in 0.05 M sodium phosphate pH 6.8 was performed on the isolated peptides to determine their molecular weights.

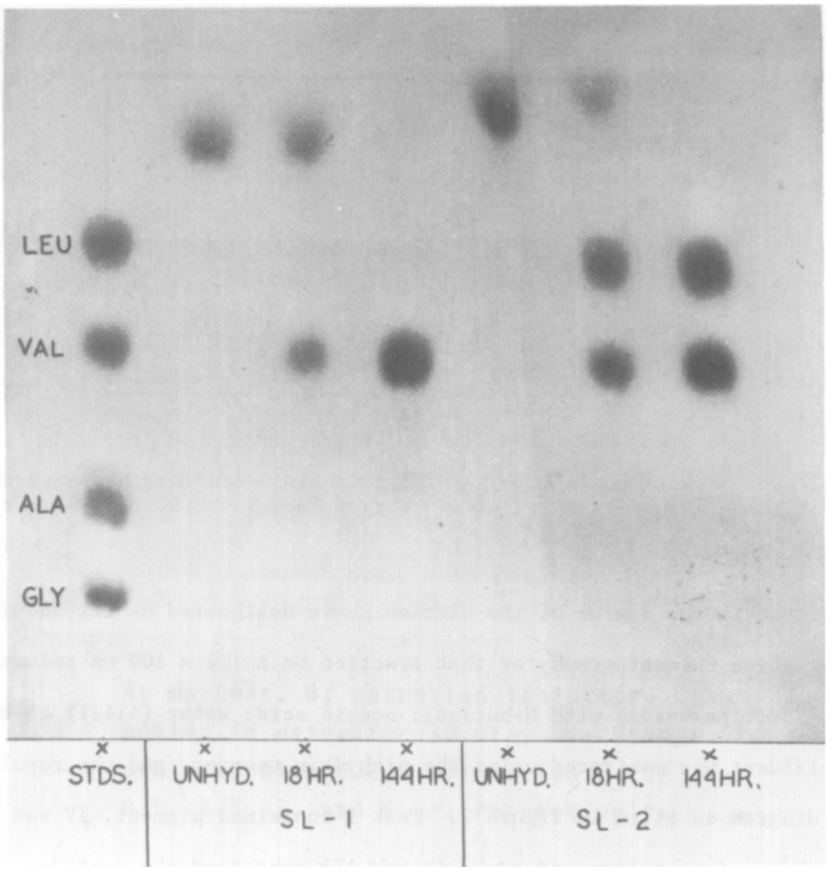

Fig. 3 Paper chromatogram of peptides isolated from elastin after 18 and 144 hours of acid hydrolysis at 100°C. Ascending, Whatman # 3MM paper, butanol, acetic acid, water 4:1:1 solvent. SL-1 is val-val, SL-2 is leu-val.

In both cases, the SL-1 and SL-2 were eluted after gly-pro-gly-gly (M.W. 286), indicating that they have molecular weights less than this marker. The compound SL-1 therefore is the dipeptide valyl-valine, while SL-2 could be either leucyl-valine or valyl-leucine. This question was resolved by the hydrazinolysis of SL-2; only valine was found in the C-terminal position, which proved that the sequence was leucyl-valine.

Both the resistance to acid hydrolysis and the decreased reaction rate with ninhydrin, appear due to a lowered accessibility of the peptide bonds to the reagents involved. This in turn may be caused by the blocking action of the hydrophobic methyl groups on the β carbon of valine in the peptides. This

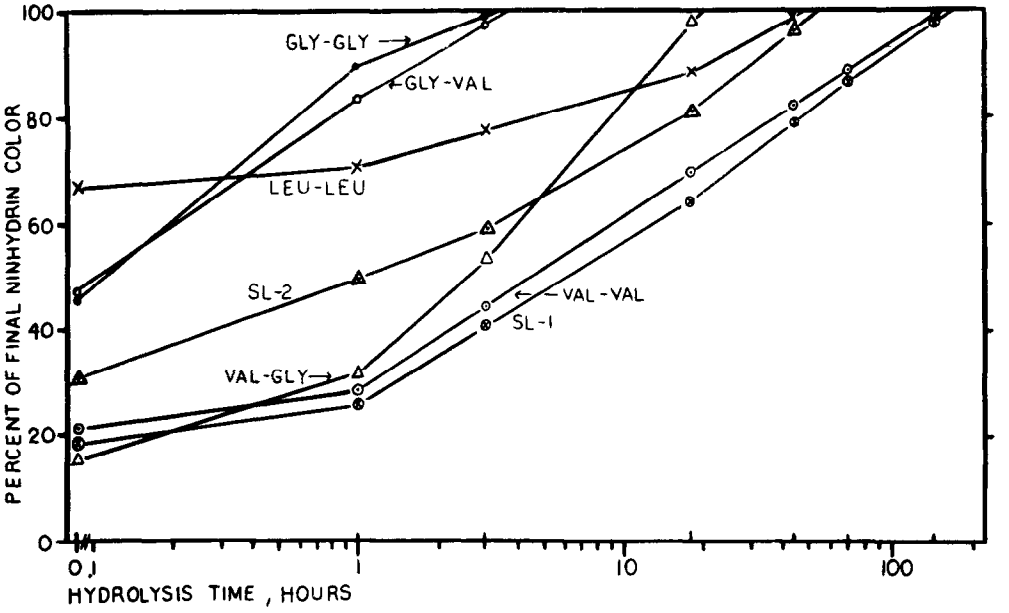

Fig. 4 Course of acid hydrolysis of some dipeptides at 100°C with 6N hydrochloric acid.

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was investigated by hydrolyzing a group of synthetic dipeptides, as well as the SL-1 and SL-2 isolated from elastin, with 6N HCl, for varying lengths of time, and by following the course of hydrolysis with the ninhydrin test (figure 4). This series of curves illustrates both the widely different resistances to hydrolysis, and the low extent of the reactivity with ninhydrin of some peptides. As expected SL-1 which has valine on both sides of the peptide bond also has greater resistance to acid hydrolysis, as well as a lower rate of ninhydrin reaction than any of the other peptides tested. SL-2, which has only a valine residue in the C-terminal position is more susceptible to both reagents. Glycyl-glycine, which has no aliphatic side chains was found to have the greatest rate of acid hydrolysis under these conditions. According to this concept it might also be expected that peptides containing isoleucine, which has a methyl group on the β carbon, would show low rates of acid hydroly sis. This was observed by Synge (1) who showed that when myokinase was hydrolyzed with acid for different lengths of time, both the valine and the isoleu-

cine were released more slowly than any of the other amino acids. In addition, it was shown by Ambler and Meadway (9) that peptides of valine and isoleucine have very low ninhydrin color values.

The important work of Partridge (10,11) has given us insight into the mechanism of cross-linking among the peptide chains of elastin, a process designed to give mechanical strength to this protein. Little knowledge has been gained, however, regarding the processes by which elastin is rendered extensible. Peptide linkages in elastin which may be partially blocked by hydrophobic methyl groups from valine or isoleucine residues, may play a role in this process. Such a masking of the carbonyl or imino group of a peptide bond may interfere with, or reduce the degree of hydrogen bonding between peptide links of adjacent turns of the same alpha helix, or between nearby peptide chains. Presumably this would reduce the rigidity of the helices and allow a certain degree of extensibility of the helical chain, with the desmosines and other cross links such as lysinonorleucine (12) acting to limit the extension, or in other ways compensate for the loss of stability caused by the extensible regions. A model of elastin containing long peptide chains having only aliphatic side groups, interrupted at intervals by polar regions with cross-linking was envisioned by Robert and Poullain (13). We have isolated from an enzymatic digest of elastin both a long nonpolar peptide large enough to be non-dialyzable with 34% of its residues as valine and a polar fraction poor in valine but enriched in the desmosine crosslinks (14). It has been suggested that the neutral, aliphatic regions of elastin are located either at the periphery of the protein (15), or in a hydrophobic shell surrounding the more polar, crosslinked regions (13). Such a model would explain both the lack of solubility of elastin in water and its resistance to common proteolytic enzymes such as trypsin and chymotrypsin, which have specificities toward peptide linkages of amino acid residues other than neutral, nonpolar ones.

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