The Titration Curves of Elastin and of the Derived α - and β -Proteins

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This paper presents titration data obtained with the preparations of native elastin from *ligamentum nuchae* of cattle and the α - and β -proteins described by Partridge, Davis & Adair (1955).

Native elastin possesses very few charged groups, and is thus characterized by a flat titration curve except in the extreme acid and alkaline range. Amino acid analysis (Partridge & Davis, 1955) reveals (in 10^5 g. protein) about 22 free carboxyl groups (aspartic and glutamic acids) and about 11 basic groups (arginine and lysine). In addition, there are about 7.5 tyrosine OH groups which will also contribute to the titration curve in the alkaline range. There are very few imidazole (histidine) groups (<0.3). These results are in fair agreement with those of the present work obtained by titration of the wet solid, which reveals about 17 acidic groups and 15–16 basic groups (up to pH 11.0).

The titration curve for native elastin (Fig. 1) is in marked contrast to those of the α - and β -proteins, both of which show the presence of many free carboxyl groups, together with an equal number of new groups titrating over the range pH 6–10 with a mean pK of 8.15. This behaviour is characteristic of α -amino groups (Cohn & Edsall, 1943).

Thus the transformation of native elastin to the soluble α - and β -proteins during treatment with hot dilute oxalic acid is accompanied by cleavage of peptide bonds with consequent decrease in the mean chain length of the molecular species present.

METHODS

Titration

A glass-electrode assembly, with a Marconi pH meter, was used to determine the pH of the solutions. The glass-electrode was free from 'sodium' error at least up to pH 11.0.

The solution to be titrated was placed in position under the glass-electrode assembly in a 50 ml. beaker and stirred by a gentle stream of nitrogen bubbles. 0.05N-HCl or -NaOH was led in through capillary tubes beneath the surface of the solution. A small drop of octanol was added to prevent frothing.

Usually, 25 ml. of solution containing 0.2–0.5 g. of protein were used for each titration, and the pH was measured after each addition of 0.5 ml. HCl or NaOH. In the case of elastin (native), the dried protein was ground to

a fine powder, which was suspended in water or 0.2M-NaCl and stirred gently with nitrogen for 15 min. before titration was begun.

Calculation of results

The results were calculated from the equation

$$\log \frac{c_2}{c_1} = pH_1 - pH_2,$$

where c_1 = concentration in g. ions/l. of H^+ (or OH^-) in the absence of the protein; c_2 = concentration in g. ions/l. of H^+ (or OH^-) in the presence of the protein; and $pH_1 = pH$ in the absence, and $pH_2 = pH$ in the presence, of the protein. The amount of H^+ bound in g. ions/l. is then given by $c_1 - c_2$. This value was subsequently corrected to give the g. ions of H^+ bound by 10^5 g. protein.

RESULTS AND DISCUSSION

Details of the preparation of native elastin powder and of the α - and β -proteins are as given by Partridge *et al.* (1955). The titration curves of these proteins are shown in Fig. 1. It is seen that the curves for elastin in water and 0.2M-NaCl respectively are very flat except at the acid and alkaline ends, whereas the curves for the α - and β -proteins are steep from pH 5.0 to 2.5 and from pH 6.0 to 10.0. The steep portion in the latter range is a unique feature of these proteins, and is not found in native proteins or in derived proteins such as gelatin (Cohn & Edsall, 1943). Analysis of

Fig. 1. Titration of purified elastin powder and the soluble α - and β -protein fractions (Partridge *et al.* 1955) derived from it by partial hydrolysis. I, β -protein; II, α -protein; III, elastin powder titrated in presence of 0.2M-NaCl; IV, elastin powder titrated without NaCl.

the curves shows that the groups responsible have a mean pK value of 8.15 and are thus probably α -amino groups. Since they behave as if they were of one species with this pK , it would be expected that half of their number would titrate between pH 8.65 and 7.65. Calculated on this basis, there are 24 present in the α -protein and 28 in the β -protein (10^5 g.). Similar values, 27 and 29 respectively, are obtained by subtracting the total H^+ bound by native elastin from the total H^+ bound by the α - and β -protein over the pH range 11.0–6.25 (the point of least slope). Thus the treatment has resulted in the production of 24–27 and 28–29 α -amino groups, in the α - and β -proteins respectively. These results are also in fair agreement with the values of 24.4 and 32.5 amino end-groups for the α - and β -proteins, obtained by the fluorodinitrobenzene technique (Partridge & Davis, 1955).

Similar analysis of the curves in the acid region shows that about 26 and 34 new acidic groups are present respectively in the α - and β -proteins. Analysis in this region is less accurate because it is not certain that all the acidic groups have been titrated at pH 2.0, which is the limit of the glass electrode. However, the comparatively good agreement between the numbers of acidic and basic groups released justifies the assumption that the former are α -carboxyl groups corresponding to the released α -amino groups.

SUMMARY

1. Titration of native elastin shows the presence (in 10^5 g. protein) of about 17 moles of acidic groups and 15–16 of basic groups. These values are in substantial agreement with those expected from the amino acid analysis.

2. The titration curves of the α - and β -proteins, derived from elastin, reveal the presence of many more acidic groups and of new groups titrating with mean pK 8.15.

3. It is shown that the new groups are α -amino in character, and that the numbers released correspond with the increase in number of acidic groups.

4. It is concluded that during the treatment of native elastin to give the α - and β -proteins, about 26 and about 31 moles of α -amino residues respectively are released in 10^5 g. of protein, with the simultaneous release of corresponding numbers of α -carboxyl residues.

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The Effect of Redox Dyes on the Active Transport of Hydrogen, Potassium and Sodium Ions Across the Yeast Cell Membrane

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Potassium ions are actively transported across the yeast cell membrane during fermentation when the external fluid contains potassium chloride. The potassium ions exchange for hydrogen ions (Conway & O'Malley, 1946; Rothstein & Enns, 1946). A similar transport of sodium ions occurs if the fermentation is carried out in the presence of 0.2M sodium citrate, without any added potassium ions (Conway & Moore, 1954). If the yeast is washed and suspended in water it slowly excretes sodium ions. These are accompanied by anions from within the cell, but if the sodium-rich yeast is suspended in 0.1M potassium chloride, the sodium is rapidly excreted in exchange for potassium ions.

The potassium and sodium ions which exchange have different carriers. Azide (0.002M) practically completely inhibits the potassium carrier but not the sodium carrier. Both are inhibited by cyanide and anoxia (Conway, Ryan & Carton, 1954). Such active transport has been interpreted as a carriage by a redox system or systems which act as the immediate energy supplier in the transport (Conway, 1951, 1953).

Apart from the immediate carriers of the ions the involvement of phosphate-bond energy at some stage in the transport of potassium ions in yeast may be inferred from the pronounced inhibitory action of 2:4-dinitrophenol (0.002M). On the other