FLUORESCENCE SPECTRA OF ARTERIAL ELASTIN

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Two unidentified fluorescent peaks have been reported in human arterial elastin, in addition to the fluorescence due to tyrosine (Blomfield and Farrar, 1965). These fluorescent peaks appear to be associated with cross-linking as the intensity is high in the insoluble residue of neonatal elastin from which a considerable portion of soluble elastin is removed during purification (Farrar et al., 1965). The activation/fluorescence spectra of these two peaks are presented, together with the effects of aging, alteration of pH, and the method of preparation of the elastin.

Although the blue-white fluorescence of elastin has long been recognized, little attempt has been made to identify the substances responsible for the fluorescence. LaBella and Lindsay (1963) found activation/fluorescence maxima at 290/340, probably due to tryptophan, at 280/315, thought to be due to tyrosine, and at 340/405, 350/440 and 370/460 mμ. The last three maxima were thought to be due to a single unidentified compound.

Andersen (1966) has studied in detail the fluorescence of resilin, the elastic protein of insects. Fluorescent compounds

separated from resilin hydrolysates by chromatography were found to have fluorescence spectra identical with dityrosine and tri-tyrosine, two compounds obtained by the enzymatic peroxidation of tyrosine.

By the use of tyrosine-C^{14} LaBella et al. (1967) have presented evidence of conversion of tyrosine into dityrosine in chick aortic elastin. The dityrosine was identified by fluorescence at 405 mμ, and by its chromatographic behaviour.

MATERIALS AND METHODS

Elastin was prepared from the pulmonary arteries of a 6 year old boy and a 52 year old man by the autoclaving-NaOH procedure previously described (Farrar et al., 1965). It was also prepared from the ascending aorta of a bullock by the same autoclaving-NaOH method, and by digesting the arteries in 88% formic acid at 45° for 2 days (Ayer et al., 1958).

20 mg. elastin was hydrolysed by 6N HCl in a boiling water bath for 1¼ hours, the HCl was evaporated off and the residue dissolved in 50 ml. buffer. The buffers used were 0.05M phosphate at pH 7.4, 0.05M citrate at pH 3.5, and 0.05M borate at pH 8.5.

Fluorescence spectra were recorded using an Aminco-Bowman spectrophotofluorimeter. The presence of activation/fluorescence maxima was determined by setting the activation wavelength at 10 mu intervals between 200 and 400 mu, and scanning the fluorescence spectra. The exact wavelength settings for activation and fluorescence maxima were determined by adjusting the activation and fluorescence wavelengths until the maximum intensity registered on the meter.

RESULTS

Three activation/fluorescence (A/F) maxima were found in each elastin hydrolysate. The spectrum of one of these, with maxima at 280/315, was identical with the spectrum of a solution of tyrosine. The other two peaks had activation maxima at approximately 250 mμ (X) and 320 mμ (Y), and both had fluorescence maxima at about 400 mμ.

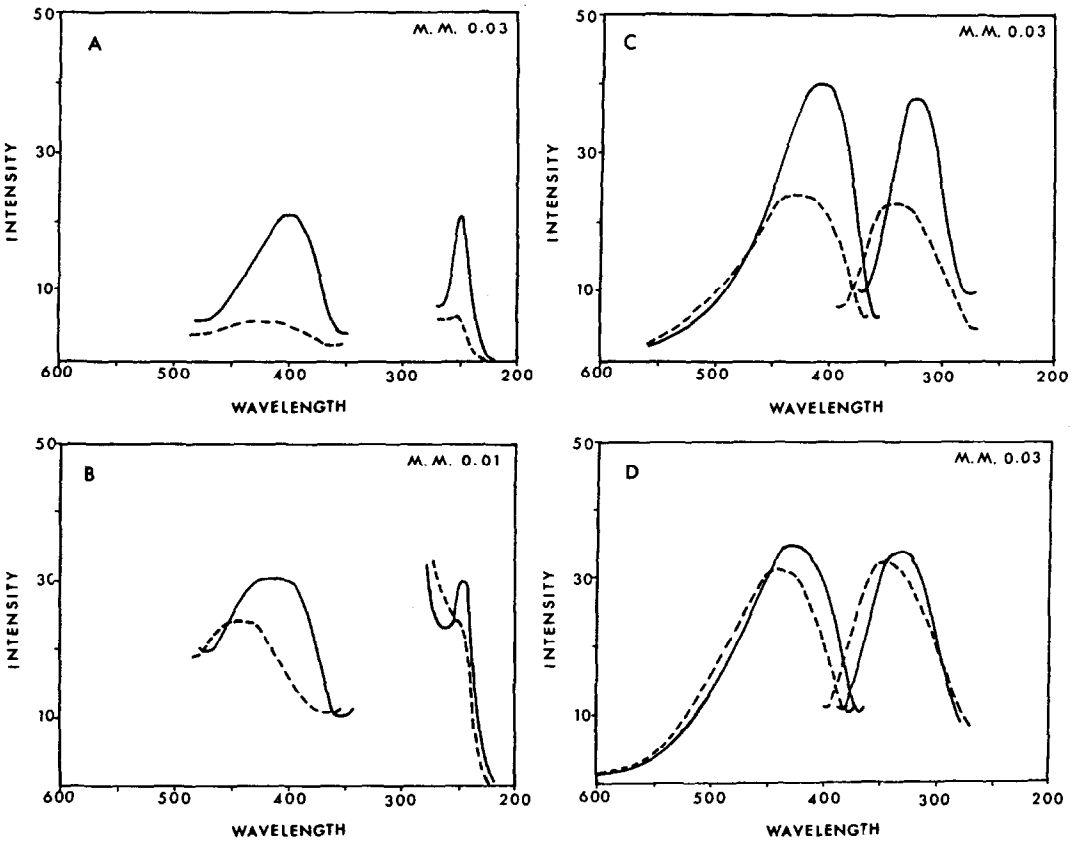

Fig. 1. The influence of age and pH on the activation/fluorescence spectra of elastin hydrolysates of human pulmonary arteries.

A. Peak X, 6 year old, ——— pH 7.4, A/F 246/392
– – – pH 3.5, A/F 250/422

B. Peak X, 52 year old, ——— pH 7.4, A/F 246/404
– – – pH 3.5, A/F 252/440

C. Peak Y, 6 year old, ——— pH 7.4, A/F 322/403
– – – pH 3.5, A/F 337/420

D. Peak Y, 52 year old, ——— pH 7.4, A/F 332/419
– – – pH 3.5, A/F 343/430

M.M. refers to the meter multiplier factor used.

At pH 7.4, the wavelengths of both peaks, X and Y, were shorter in the child than in the adult. The wavelength maxima of peak X increased from 246/392 in the child (Fig. 1A) to 246/403 in the adult (Fig. 1B), and of peak Y from 322/403 in the child (Fig. 1C) to 332/419 in the adult (Fig. 1D).

With buffer of pH 8.5, the wavelengths of peaks X and Y were unaltered, but the intensity of fluorescence increased in all samples.

At the acid pH of 3.5, there was an increase of both activation and fluorescence wavelengths and a decrease in the intensity of the fluorescence with both X and Y (Fig. 1A-D).

In the bullock's arteries, the fluorescence at pH 7.4 of elastin prepared by formic acid isolation compared with NaOH-prepared elastin, showed a decrease in intensity and increase

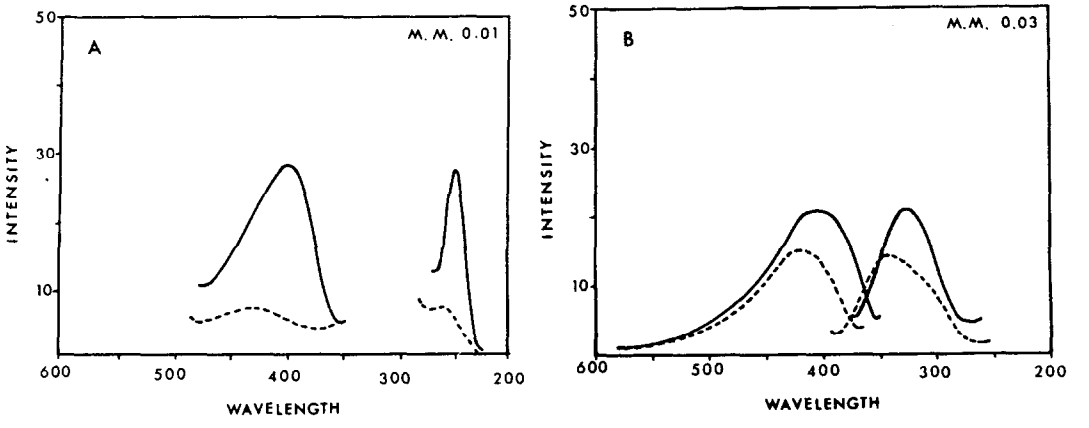

Fig 2. The effect of NaOH and formic acid isolation of elastin on the activation/fluorescence spectra of elastin hydrolysates from the ascending aorta of a bullock.

A. Peak X ——— NaOH-isolated elastin, A/F 247/395
- - - Formic acid-isolated elastin, A/F 251/420

B. Peak Y ——— NaOH-isolated elastin, A/F 328/405
- - - Formic acid-isolated elastin, A/F 344/425

M.M. refers to the meter multiplier factor used.

in wavelength. The A/F maxima for peak X (Fig. 2A) were 247/395 by NaOH isolation and 251/420 by formic acid extraction; and for peak Y (Fig. 2B), the corresponding wavelengths were 328/405 for NaOH and 344/425 for formic acid preparations.

DISCUSSION

By ion-exchange chromatography, Andersen (1966) has isolated two compounds from resilin which have been named Compounds I and II, and which have fluorescence spectra identical with trityrosine and dityrosine respectively. In alkali, Compound II and dityrosine have A/F maxima at 254/413, which agree well with peak X. At pH 7.7, Compound I has activation peaks at 255 and 320 mμ, which correspond to the activation wavelengths of X and Y. The intensity of fluorescence at 320 mμ is two to three times the intensity at 255 mμ, which again agrees with peaks X and Y. However, at pH 3.4, the 255 maximum of Compound I decreases slightly to 250, whereas in the child, peak X increases slightly from 246 to 250 mμ; and, further, with Compound I the 320 maximum decreases quite markedly to 285 mμ, which contrasts with the increase in peak Y from 322 to 337 mμ in the child. From these results it appears that the fluorescence spectra of elastin hydrolysates correspond with the fluorescence spectra of dityrosine and trityrosine at neutral and alkaline pH, but not at acid pH.

Preparation of elastin by formic acid extraction results in a decrease in elastin fluorescence intensity and shift of the wavelength values to higher levels similar to the effect produced by redissolving the elastin hydrolysates of NaOH-prepared elastin in buffer of pH 3.5. The explanation for this change has not yet been determined.

Aging produces an increase in wavelength similar to that produced by an acid buffer or by formic acid preparation. A

detailed analysis of the effects of age on the intensity and wavelength of fluorescence will be presented in a future publication (Blomfield and Farrar, In preparation). Isolation of the fluorescent compounds in elastin and of adjacent amino acids will help to determine whether the age change is produced by such factors as a changing amino acid environment or increasing cross-linking as in the conversion of dityrosine to polytyrosines.

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