

resolved emission spectra that we measured were particularly helpful in analysing and assigning the nature of the emission doublet to any greater extent than was possible from steady state measurements and quenching data.)

3.2 Thermal stability of the native structure

We next investigated the structural stability of δ -crystallin by studying its denaturation properties. First we studied the thermal denaturation profile of the protein by monitoring the temperature dependent changes in its fluorescence spectrum. The native protein displays an emission doublet at 315 and 325 nm as seen above, while the denatured protein does so around 350 nm (in GuHCl), as described below.

Figure 3a shows a sharp change in the wavelength of the emission maxima of δ -crystallin upon heating. The 315, 325 nm emission doublet, characteristic of the native protein, is retained until around 60°C, at which point it coalesces and shifts into a single band centred around 330 nm. This value suggests that the protein is not denatured yet, but maintains a tertiary structure in which the Trp residues are in a non-polar environment. Unfortunately the solution becomes turbid and the protein precipitates beyond 60°C, with an apparent T_m of 60.2°C. It is apparent however, that the fluorescence band is still in the 330 nm region even around 91°C. These results suggest that the tertiary structure of δ -crystallin is resistant to heat even around 91°C, unlike γ -crystallin (the core protein of the mammalian lens), which denatures around 72°C (Kono *et al* 1990). The behaviour of δ -crystallin is akin to that of the cortical lens protein α -crystallin, which is also multimeric, and which does

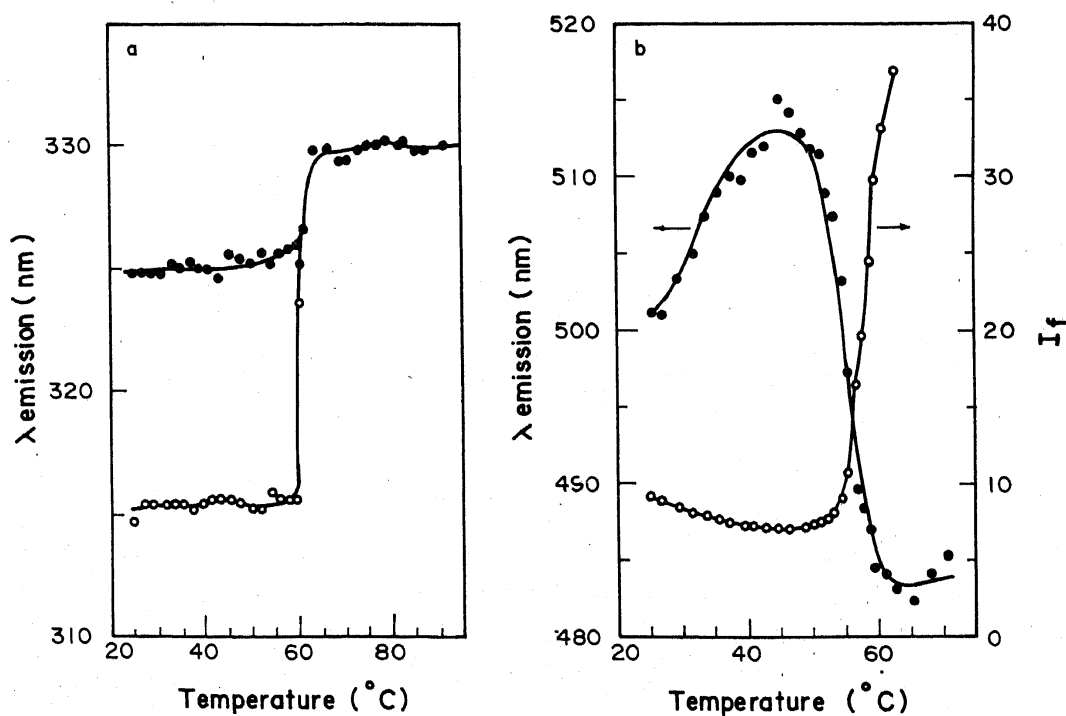


Figure 3. Changes in the fluorescence properties of δ -crystallin upon heating: (a) emission maximum of the Trp doublet, 315 nm (○) and 325 nm (●); (b) emission maximum (●) and relative intensity (○) of externally added fluorescence probe ANS (250 μ M ANS and 0.6 mg/ml protein in pH 7.0 phosphate buffer). I is intensity in arbitrary units.