# Behaviour of $\alpha$-elastin in bulk and at aqueous surfaces

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

..... (Amanda R. Lindsay)

‘But I should like to know-’ Pippin began

‘Mercy!’ cried Gandalf. ‘If the giving of information is to be the cure of your inquisitiveness, I shall spend all the rest of my days in answering you. What do you wish to know?’

‘The names of all the stars, and of all living things, and the whole history of Middle-earth and Over-heaven and of the Sundering Seas,’ laughed Pippin. ‘Of course. What less? But I am not in a hurry tonight’

Exchange between Peregrin (Pippin) Took and Gandalf the White from ‘The Two Towers - The Lord of the Rings Part 2’ By J.R.R. Tolkien

# Abstract

The purpose of this work was to examine the behaviour of the soluble elastin derivative, $\alpha$-elastin, under a variety of conditions. Although studies of $\alpha$-elastin in solution have been made, confining the protein molecules to a two dimensional state in a monolayer allows probing of different conformational states.

Bulk viscometry experiments indicated, consistent with previous work, that $Ca^{2+}$ affects $\alpha$-elastin differently to $Na^{+}$. The intrinsic viscosity of $\alpha$-elastin in water was 0.0073 mL/mg at room temperature and it was seen to increase with decreasing temperature. In 0.1 M calcium chloride it was seen that the radius of gyration of the elastin increased by 6% with a 17°C rise in temperature, whereas in water and 0.1 M sodium chloride the increase was only 2%.

When confined to the surface it was demonstrated that $\alpha$-elastin monolayers on water behave viscoelastically in the surface pressure range 12-20 mN/m , viscoelastic behaviour was also seen on 0.1 M $CaCl_2$ in the surface pressure range 14-18 mN/m. Examination of the dissipative component of the complex modulus showed phase transition occurring between 8 and 10 mN/m on both water and calcium chloride subphase. From the value of the dissipative component below the phase transition, the transition was identified as semi-dilute to concentrated. Fitting to Eyring's model allowed calculation of the area per segment in motion of the $\alpha$-elastin on water $A_m = 48$ Å$^2$/segment, and on calcium chloride $A_m = 76$ Å$^2$/segment. These values are consistent with calculated areas per molecule which indicate that at a given surface pressure an $\alpha$-elastin molecule on calcium chloride takes up at least 2.4 times are much space as a molecule on water.

Quasi-static compressions and extensions of $\alpha$-elastin monolayers were carried out on three different subphases at three different values of pH. This gave the surface pressure - area isotherm for each. Dilational modulus calculation indicated a phase

transition at around 8 mN/m. Below 5 mN/m the monolayer was in the semi-dilute regime, between good and $\Theta$ solvent conditions, the Flory exponent, $\nu = 0.671 \pm 0.002$. This indicated that the observed phase transition is semi-dilute to concentrated which confirms the surface viscometry results. The salt solutions were seen to provide the $\alpha$-elastin with conditions closer to good solvent with $\nu_{NaCl} = 0.70 \pm 0.04$. and $\nu_{CaCl_2} = 0.7 \pm 0.2$.

Fitting an exponential to the decay of surface pressure at constant area indicated that time constant for the decay was consistent for all three subphases, $\tau = 0.001 \pm 0.0003$ s. This consistency shows that the relaxation of the monolayer is not limited by electrostatic interactions between the monolayer and the subphase.

By altering the temperature of the subphase it was seen that $\alpha$-elastin forms monolayers up to 40°C, which is above the temperature at which bulk solutions of $\alpha$-elastin coacervate and drop out of solution. Thus, coacervation in two dimensions does not occur under the same external conditions as in three dimensions.

By examining the pressure at the surface of very dilute solutions of $\alpha$-elastin it was seen that monolayers did not spontaneously form at a water surface However, with stirring a monolayer formed quickly which resulted in a surface pressure rise of 15 ± 2 mN/m. When $\alpha$-elastin was added below a lipid monolayer and stirred it was seen that it was able to insert at surface pressure above 30 mN/m. Using fluorescently labelled lipids it was seen that the label did not affect the elastin insertion into the lipid monolayer. It was seen that the $\alpha$-elastin insertion into a PC:NBD-PC monolayer disrupted the domain structure of the monolayer.

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