

The effect of actin filament compliance on the interpretation of the elastic properties of skeletal muscle fibres

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Summary

Recently, X-ray diffraction studies provided direct evidence for an appreciable length change in the actin filament upon activation. This finding has profound implications on the interpretation of the elastic properties of skeletal muscle fibre. In this study we determined the compliance of the actin filament during activation, using the data obtained previously from quick stretch and release experiments on skeletal muscle fibres of the frog. The effects of filament compliance are demonstrated clearly in the elastic properties of partially activated fibres. The low-frequency elasticity increases linearly with tension, reflecting an increase in the number of force-producing cross-bridges. At higher frequencies, this linearity is lost. In this study we describe the data consistently in terms of a cross-bridge stiffness increasing linearly with tension and a constant Young's modulus for the actin filament of 44 MN m^{-2} . This corresponds to a compliance of 23 pm i m^{-1} per kN m^{-2} tension developed. Using this value for the actin filament Young's modulus, its contribution to the elastic properties of skeletal muscle fibre of the frog is considered in rigor and relaxation. The filament compliance hardly affects the overall elasticity of the muscle fibre in relaxation. In contrast, it contributes to a large extent to the overall elasticity in rigor. Taking account of the filament compliance, we find that the Young's modulus in rigor exhibits an increase from 14 MN m^{-2} at frequencies below 500 Hz to 55 MN m^{-2} above 40 kHz.

Introduction

The elastic properties of muscle fibres are generally interpreted in terms of the number of cross-bridges between the myosin and actin filaments and their individual elasticity (Kawai & Brandt, 1980; Ford *et al.*, 1981). Recently, X-ray diffraction studies provided direct evidence for an appreciable length change of the actin filament of about 2–3 nm per half sarcomere upon maximal activation (Huxley *et al.*, 1994; Wakabayashi *et al.*, 1994). As pointed out by Goldman and Huxley (1994) this result has profound implications for the interpretation of mechanical experiments. Indeed, some earlier mechanical studies indicated a sizeable filament compliance (Bagni *et al.*, 1990; Jung *et al.*, 1992).

In this study we determined the compliance of the actin filament using the data obtained previously from quick stretch and release experiments on skeletal muscle fibres of the frog in activation. The implications of the filament compliance are conveniently demonstrated in the elastic properties

of fibres at a range of activation levels. The elastic properties of the half sarcomere were modelled by three damped and one undamped elastic component in series (Blangé & Stienen 1985; Jung *et al.*, 1988). Thus, for each level of activation four elastic constants and three time constants (or alternatively damping constants) fully describe the Young's modulus of the muscle fibre from which the stiffness of the half sarcomere can be derived. When plotted as a function of the isometric tension, the elastic constants corresponding to slower components of the tension recovery increased linearly with the developed tension (see Fig. 2., Z_3 and Z_4). In contrast, the elastic constants corresponding to the instantaneous recovery exhibited a strongly non-linear behaviour (see Fig. 2., Z_1 and Z_2).

Here, we analysed the data in terms of a compliance of the actin filament and cross-bridge compliance, using a ladder structure to model the overlap zone of the sarcomere (A-band) similar to the structures proposed by White and Thorson (1973) and Ford and colleagues (1981) (Fig. 1). The

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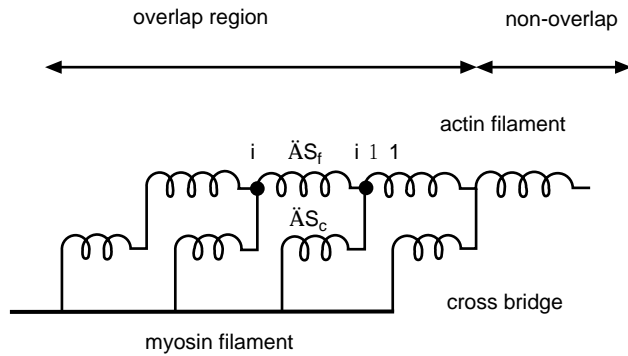


Fig. 1. A schematic representation of the elastic elements in the sarcomere. The actin filament is modelled as a pure elasticity. The cross-bridges in the overlap zone are modelled by visco-elastic elements.

non-overlap zone (I-band) is represented by an additional elasticity of the actin filament in series with the overlap zone. The stiffness corresponding to the cross-bridges increases linearly with the developed tension (Ford *et al.*, 1981). When both the overlap and non-overlap zones are taken into account, a good fit to the data is obtained using a filament elasticity of 44 MN m^{-2} , corresponding to a compliance of $23 \text{ pm} \cdot \text{m}^{-1}$ per kN m^{-2} tension.

We use this model for analysing the elastic properties of skeletal muscle (*M. Iliofibularis*) of the frog (*Rana esculenta*) as determined in an earlier study using quick stretch and release experiments (Jung *et al.*, 1988). In particular we are interested in extracting the cross-bridge properties from the overall characteristics of the fibre. The analysis shows that the Young's modulus of the relaxed muscle fibre is almost completely determined by the cross bridges. In contrast, the filament elasticity has substantial influence on the Young's modulus of the rigor muscle.

Materials and methods

In earlier studies the elastic properties of the muscle fibre have been extracted from the tension response to a rapid stepwise length change, using a model in which the fibre was regarded as a uniform cylindrical rod, which can be subdivided into small identical units (half sarcomeres) (Blangé & Stienen 1985; Jung *et al.*, 1988; De Winkel *et al.*, 1993). The elastic properties of a single unit were modelled by an undamped and two or three damped elastic elements in series. Thus up to four elastic and three time constants define the Young's modulus from which the stiffness of the half sarcomere can be derived

$$S = \frac{ZA}{l_{hs}} \quad (1)$$

with S the stiffness, Z the Young's modulus, A the cross-sectional area and l_{hs} the length of the half sarcomere. In

this study we model the sarcomere using a ladder structure for the overlap zone and an additional elasticity for the non-overlap zone. This model is similar to the structures proposed by White and Thorson (1973) and Ford and colleagues (1981) (Fig. 1). Theoretically, the filament compliance can be expected to reside in both the actin and the myosin filaments. In this case a similar approach can be used, resulting in an additional series elasticity related to the lesser compliant filament. However, since X-ray diffraction studies primarily point to a substantial compliance in the actin filament and not in the myosin (Huxley *et al.*, 1994; Wakabayashi *et al.*, 1994), we will only consider the compliance of the actin filaments and the cross-bridges.

The overall Young's modulus Z_T ($= 1/C_T$, with C_T the overall normalized compliance) of a muscle fibre can be expressed in terms of the Young's modulus in the overlap and the non-overlap zones of the sarcomere.

$$\frac{1}{Z_T} = \frac{\dot{a}}{Z_{non-overlap}} + \frac{(1-\dot{a})}{Z_{overlap}} \quad (2)$$

with \dot{a} the length of the non-overlap zone divided by the half-sarcomere length. The Young's modulus of the actin filament, Z_f , fully defines the Young's modulus in the non-overlap zone of the sarcomere, when additional series compliances from e.g. the attachments of the fibre are negligible. In the overlap zone the contributions of both the filaments Z_f and the cross-bridges Z_c are taken into account (Fig. 1). The full derivation of this expression is given in the appendix.

$$Z_{overlap} = Z_f^a \left(\frac{e^a - e^{-a}}{e^a + e^{-a}} \right) \quad (3)$$

with:

$$a = \sqrt{\frac{Z_c}{Z_f}} \quad (4)$$

In case the Young's modulus of the cross bridges is much smaller than that of the actin filament, this relation simplifies to:

$$Z_{overlap} = Z_c \quad (5)$$

On the other hand when the Young's modulus of the cross bridges is much larger than that of the actin filament, we find:

$$Z_{overlap} = \sqrt{Z_c Z_f} \quad (6)$$

The Young's modulus of the cross-bridges Z_c reflects the elastic properties of the attached cross bridge as well as its binding. This can be modelled by a combination of undamped and damped elastic elements. For the sake of simplicity, we assume the actin filament to behave as an undamped linear elastic component.

Results

Partially activated muscle fibres

At first, the elastic constants of partially activated skeletal muscle fibres (*M. Iliofibularis*) of the frog (*R. esculenta*) will be considered. These four constants

were determined in an earlier study (Jung *et al.*, 1992) from quick stretch and release experiments with single fibres, skinned by freeze drying (Stienen *et al.*, 1983; Stienen & Blangé, 1985). The fibres were mounted isometrically in the dry state between the loud-speaker motor and the home-made force transducer (De Winkel *et al.*, 1993) with \AA -cyanoacrylate glue (Cyanolit 202, 3M Center, St Paul, MN). As the compliance of the glued ends is negligible, a sharp interface is obtained between the fibre ends and the functional segments. The composition of the solutions used for inducing relaxation or (partial) activation were calculated using a computer program based on that of Fabiato and Fabiato (1979). Their composition is given in Jung and colleagues (1992). The experiments were carried out at a sarcomere length of 2.15 μm , and a temperature of 48 C.

The elastic constants were determined by fitting the first 2 ms of the tension transient after a rapid stepwise length change to the model containing one undamped and three damped elastic components in series (Jung *et al.*, 1988). The elastic constant Z_1 corresponds to the Young's modulus at some frequency ν above 40 kHz. The constants Z_2 , Z_3 and Z_4 correspond to the Young's modulus at roughly 8 kHz, 2 kHz and 100 Hz respectively. Now, the model for the filament compliance will be used to disentangle the contributions of the actin filament and the cross-bridges. The data are fitted to the model using the non-linear least squares Marquart procedure (ZXSSQ) from the IMSL library (Visual Numerics Inc., Houston TX).

We assume the number of cross bridges to increase linearly with tension T (Ford *et al.*, 1981) with a frequency-dependent slope α_i . This is consistent with a linear increase of the Young's modulus attributable to the cross bridges. However, the four elastic constants exhibit an appreciable value at zero tension (Fig. 2). This rest elasticity $Z_{0,i}$ can be interpreted in two ways. In the first interpretation, this elasticity reflects weakly bound cross bridges, that are incapable of producing force. Following this interpretation, their contribution must be added to the Young's modulus of the cross bridges so that:

$$Z_{c,i} = Z_{0,i} + \alpha_i T \quad (7)$$

for $i = 1-4$. As the number of weakly bound cross-bridges is very small (Jung *et al.*, 1989), we ignore a possible reduction of weakly bound bridges upon activation. Based on the elastic constant Z_4 , corresponding to the Young's modulus around 100 Hz, we find that the filament Young's modulus must be larger than 33 MN m^{-2} . A lower value would introduce a curvature in the relation $Z_4(T)$, which is not observed experimentally. On the other hand,

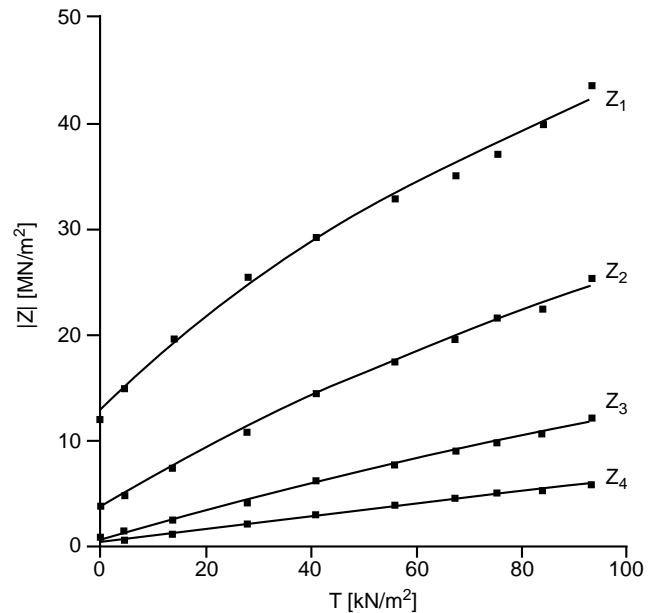


Fig. 2. The four apparent elastic constants Z_1 – Z_4 necessary to describe the first 2 ms of the tension responses of Ca^{2+} activated fibres as a function of the isometric tension T (data from Jung *et al.*, 1992). The solid lines are the fit to the data assuming a constant Young's modulus for the actin filaments of 44 MN m^{-2} , and parameters for the cross-bridge elasticity as given in Table 1.

the relation $Z_1(T)$, corresponding to frequencies higher than 40 kHz, is clearly non-linear. We find an upper limit of 70 MN m^{-2} , corresponding to 1.5 times the minimum value for the summed squared residuals. The best fit of the elastic constants for all four frequencies was obtained with a Young's modulus for the actin filament of $Z_f = 44 \text{ MN m}^{-2}$ corresponding to a compliance of 23 $\text{pm } \mu\text{m}^{-1}$ per kN m^{-2} tension (sarcomere length 2.15 μm , overlap fraction $\dot{a} = 0.25$). The values for the rest elasticities $Z_{0,i}$ and for α_i are given in Table 1.

A second explanation for the rest elasticity is that it resides in passive structures either parallel to the sarcomere or between the myosin filament and the Z-line. In this case this contribution must simply be added to the Young's modulus of the overlap zone. The best fit to the data now yields $Z_f = 40 \text{ MN m}^{-2}$ (compliance 25 $\text{pm } \mu\text{m}^{-1}$ per kN m^{-2} tension).

Table 1. The parameters describing the cross-bridge elastic constants corresponding to the fit in Fig. 2. Note that the slope α_i is dimensionless.

i	$Z_{0,i} [\text{MN m}^{-2}]$	α_i
1	11.2	505
2	2.8	245
3	0.6	106
4	0.2	52

In both interpretations we take account of the compliance of the non-overlap zone and the overlap zone separately. They yield good fits to the data and the value of the filament compliance corresponds very well to the values found by Huxley and colleagues (1994) and Wakabayashi and colleagues (1994). In contrast, when the filament compliance in the overlap zone is ignored, i.e. $Z_{overlap} = Z_c$, we find a reasonable fit to the data only with a Young's modulus in the I-band of $Z_{non-overlap} = 22 \text{ MN m}^{-2}$, (compliance $45 \text{ pm } \dot{\text{m}}^{-1}$ per kN m^{-2} tension). When the compliance in the non-overlap zone is neglected we find $Z_f = 24 \text{ MN m}^{-2}$ (compliance $41 \text{ pm } \dot{\text{m}}^{-1}$ per kN m^{-2} tension). These values don't correspond with the results from X-ray diffraction studies (Huxley *et al.*, 1994; Wakabayashi *et al.*, 1994). Therefore, we believe that the filament compliance must be taken into account in both zones of the sarcomere.

The Young's modulus of cross-bridges in relaxation and rigor

The prime target of quick stretch and release experiments is usually the characterization of the number of cross-bridges in the muscle fibre and their elastic properties. Assuming the elastic properties of the actin filament don't depend on the concentration of ATP or Ca^{2+} present in the fibre, the model given above can be used to extract the cross-bridge properties reflected in Z_c from the experimentally obtained Young's modulus Z_T . We use the Young's modulus for the actin filament $Z_f = 44 \text{ MN m}^{-2}$ as found above. In addition, we extract Z_c assuming an infinitely large value for Z_f , corresponding to a non-compliant actin filament. Both the overlap and the non-overlap zones are taken into account.

The elastic constants of skeletal muscle fibre (*M. Iliofibularis*) of the frog (*R. esculenta*) were determined in a separate study in rigor and relaxation by fitting the tension response to a fast length change to a model containing one undamped and two damped elastic components in series (Jung *et al.*, 1988). The Young's modulus Z_T is calculated as function of the frequency ν from these parameters using equation 6 in Jung and colleagues (1988). From these values for Z_T we extracted the Z_c as a function of ν . The result is plotted in Fig. 3 (solid lines). The dashed lines reflect the Young's modulus of the muscle fibre in rigor and relaxation, which equals $(1 - \dot{a})Z_c$ assuming no filament compliance.

In the relaxed fibre we find that the observed Young's modulus Z_T is almost fully determined by the cross-bridges. Obviously, when the cross-bridges are very compliant (over 1000 pm per half sarcomere per kN m^{-2} tension in a relaxed fibre), the additional contribution of the actin filaments only makes a small difference. On the other hand, it

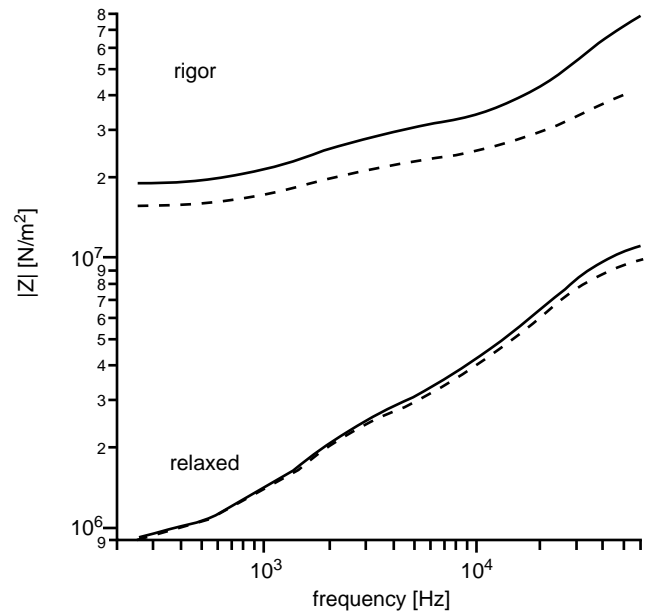


Fig. 3. The magnitude of the complex Young's modulus $Z = (1 - \dot{a})Z_c$ reflecting the cross-bridge properties, Z_c of skeletal fibre of the frog in rigor and relaxation (data from Jung *et al.*, 1988, Table 2). The dashed lines represent $Z = Z_T$ assuming no filament compliance. The solid lines represent Z assuming a Young's modulus for the actin filament of 44 MN m^{-2} .

cannot be ignored under rigor conditions. Upon including the filament compliance in the interpretation of the observed Young's modulus, we find the Young's modulus reflecting the cross-bridge properties, Z_c , to increase sharply. At the low-frequency end we find a value of 14 MN m^{-2} , whereas a value of 12 MN m^{-2} is found assuming no filament compliance. At frequencies above 40 kHz we find $Z_c = 55 \text{ MN m}^{-2}$ instead of the 31 MN m^{-2} when the filament compliance is ignored.

Discussion

The elastic properties of partially activated fibres are described consistently in terms of the Young's modulus of the cross-bridges Z_c and of the actin filaments Z_f . The latter is taken independent of the developed tension and of the frequency. This reflects the fact that the filaments comply instantaneously to a length change without exhibiting additional viscous behaviour. In contrast, the Young's modulus Z_c depends on the frequency of the length modulation because of the visco-elastic behaviour of the cross-bridges. In partially activated fibres it is taken to increase linearly with the developed tension over the full frequency range, in agreement with the view that the number of cross bridges is proportional to the developed tension. This was suggested earlier by Ford and colleagues (1981), however assuming a

negligible compliance in the filaments. In this study we show that the compliance of the filaments does not affect the linearity at lower frequencies, whereas the non-linearity observed at higher frequencies is consistent with a proportional number of cross-bridges when the filament compliance is accounted for. When the actin filament elasticity is taken into account in both the overlap and the non-overlap zones of the sarcomere, we find a value $Z_f = 44 \text{ MN m}^{-2}$ (compliance $23 \text{ pm} \cdot \text{m}^{-1}$ per kN m^{-2} tension) when a rest elasticity in relaxed fibres is attributed to weakly bound cross-bridges and $Z_f = 40 \text{ MN m}^{-2}$ (compliance $25 \text{ pm} \cdot \text{m}^{-1}$ per kN m^{-2} tension) when this rest elasticity is explained in terms of passive structures parallel to the sarcomere. These values correspond well to the result of Huxley and colleagues (1994) and Wakabayashi and colleagues (1994) and show a good agreement with the values obtained by Higuchi and colleagues (1995) under rigor conditions.

Based on the data from partially activated fibres, we don't favour one interpretation over the other. However, a closer inspection of the relaxed state shows that the apparent elastic constants for relaxed muscle fibres increase substantially when the ionic strength is decreased (Jung *et al.*, 1989). This is commonly associated with an increase in the number of weakly bound cross-bridges. Indeed, the trend of the elastic constants with ionic strength suggests that even at 160 mM some weakly bound cross-bridges are present. This backs the hypothesis that the rest elasticity is related to these weakly bound cross-bridges.

Whereas the absolute value of the actin filament compliance corresponds remarkably well with that found in other studies, the comparison of the relative contribution of the actin filament compliance to the overall compliance is more cumbersome. This relative contribution depends strongly on specific experimental conditions such as the temperature, the sarcomere length and the frequency of the length modulations. For the fully activated fibres we find that about 44% of the high frequency compliance (above 40 kHz) is caused by the actin filaments, whereas at the low frequency end (below 100 Hz) this fraction drops to 10% due to the slower components in the compliance of the cross bridges. The values reported in other studies lie well within this range (Julian & Morgan, 1981; Bagni *et al.*, 1990). Nevertheless, the fraction of 10% at low frequencies points to a substantial compliance of the cross bridges. This may be attributed to the particular muscle preparation and the low temperature at which the experiments were carried out. Furthermore, at smaller sarcomere lengths, the contribution of the I-band (non-overlap zone) becomes less prominent.

After correction for the filament compliance the Young's modulus attributable to the cross-bridges in the relaxed fibre exhibits a gradual increase with frequency almost over the full frequency range. This can be interpreted in terms of attachment and detachment of weakly bound cross-bridges. Remarkably, the large frequency range points to an equally large range of rate constants involved. The Young's modulus corresponding to the rigor fibre also increases with the frequency. As suggested in previous studies, we believe that this frequency characteristic points to transitions of the cross bridges between distinct strongly bound states (De Winkel *et al.*, 1994). Interestingly, the correction for the filament compliance makes this effect even more prominent.

Recently, the filament compliance was determined in skeletal muscle under rigor conditions (Higuchi *et al.*, 1995). The compliance was measured at various tension levels by adjusting the fibre length. On all cross-bridges these length adjustments cause a change in stress and consequently a change in the average force produced. A significant stretch dependence of the overall compliance was observed in the tension range between 0 and 100 kN m^{-2} . This was at least partially attributed to the actin filament. Surprisingly, we find no evidence for such a stretch dependence in the same tension range, when measured in partially activated fibres. In our study, the tension level was varied by changing the level of activation and consequently the number of identical cross bridges. The linear relation between the cross-bridge stiffness (Z_c) and the tension supports this view.

The discrepancy between the results from rigor and partially activated fibres may point to an influence of ATP or calcium on the actin filament properties. However, another option is to attribute the stretch dependence to the strongly bound cross bridges. Indeed, in a separate study, we found evidence for the latter. The time-resolved Young's modulus was determined during a length change of up to 4 nm per half sarcomere in the frequency range from 250 Hz to 10 kHz. At frequencies below 1 kHz a 20% increase was found per nm/half sarcomere stretch. Above 1 kHz this stretch dependence diminished to about half the value at 10 kHz (Van der Heide *et al.*, 1996). Importantly, in Fig. 3 it is shown that the influence of the actin compliance becomes more visible towards the higher frequencies, where the overall Young's modulus is high. Any non-linearity in the actin filament elasticity should therefore show up most clearly at high frequencies. As we found precisely the opposite, we believe that the stretch dependence of the elasticity of the rigor fibre must be attributed to the cross bridges rather than the actin filament.

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Appendix

The stiffness of the ladder structure in the overlap zone at point $i + 1$ can be expressed in terms of the stiffness at point i in series with the contribution of the actin filament $\sim S_f$ over a length $\sim x$ and this total structure parallel to the cross-bridge contribution $\sim S_c$ over the segment $\sim x$:

$$S_{i+1} = \left(\frac{1}{S_i} + \frac{1}{\sim S_f} \right)^{-1} + \sim S_c \quad (\text{A1})$$

Defining the incremental stiffness $\sim S = S_{i+1} - S_i$ Equation A1 can be rewritten as:

$$\sim S(S_i + \sim S_f) = -S_i^2 + S_i \sim S_c + \sim S_f \sim S_c \quad (\text{A2})$$

When the cross-bridge density along the actin filament is constant, its contribution to the stiffness $\sim S_c$ is proportional to a segment length $\sim x$ as $\sim S_c = c_c \sim x$ and c_c a stiffness per unit length of actin. It is reasonable to assume that this condition is met when the cross-bridges are free to attach and detach from the actin filament. This is the case in activation and relaxation. The compliance of the actin filament is proportional to the segment length $\sim x$. Thus, $1/\sim S_f$ is proportional to $\sim x$ as $1/\sim S_f = c_f \sim x$, with c_f a reciprocal stiffness per unit length of actin. Now Equation A2 can be rewritten as:

$$\frac{\sim S}{\sim x} \left(S_i \sim x + \frac{1}{c_f} \right) = -S_i^2 + S_i c_c \sim x + \frac{c_c}{c_f} \quad (\text{A3})$$

Taking the limit $\sim x \rightarrow 0$, with c the ratio c_c/c_f we find:

$$\frac{dS}{c - S^2} = c_f dx \quad (\text{A4})$$

$$Z = \sqrt{Z_f Z_c} \tanh \sqrt{\frac{Z_c}{Z_f}} \quad (\text{A6})$$

Upon integration from $x = 0$ to $x = l$ we find:

$$S = \sqrt{c} \tanh(l \cdot c_f \sqrt{c}) \quad (\text{A5})$$

or after substitution of $a = \sqrt{Z_c/Z_f}$:

Upon normalization for length and cross section we find:

$$Z = Z_f^a \left(\frac{e^a - e^{-a}}{e^a + e^{-a}} \right) \quad (\text{A7})$$