Orientation of skeletal muscle actin in strong magnetic fields

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Measurement of birefringence is used to follow actin filament and paracrystal formation in a strong magnetic field. Both F-actin and paracrystals orientate parallel to the field. This confirms that globular proteins arranged in filamentous assemblies can orientate in magnetic fields. This is consistent with the α-helical component of the actin subunits being approximately aligned along the actin filament.

Actin *Magnetic orientation* *Birefringence* *α-Helix* *Muscle contraction*

1. INTRODUCTION

Strong magnetic fields can be used to obtain highly orientated specimens of biological material which are suitable for structural analysis using optical [1–3], diffraction [2–9] and NMR [10,11] techniques. As magnetic anisotropy is conformation-dependent structural information can be deduced from the orientational behaviour of particles [2,3,12–14]. Also, studies of polymerisation and assembly of proteins such as fibrin can be accomplished by monitoring the birefringence in a magnetic field [3,7]. It has been clearly demonstrated that aggregates of fibrous protein may be magnetically orientated especially if they include a significant percentage of aligned α-helix as in fibrin [3,8] or myosin tails isolated from muscle [9] where the orientation of the α-helix is parallel to the direction of the applied field. Whole muscle fibres orientate with their long axis parallel to magnetic fields [15]. This is presumably mainly due to the arrangement in the fibres of myosin in muscle thick filaments and tropomyosin in thin filaments which are proteins known to contain large amounts of α-helix. However, it is not clear if polymers of globular proteins like actin, which is the main component of the thin filament, can be orientated by magnetic fields.

Here we investigate the behaviour in a strong magnetic field of actin, a globular protein that contains about 30% α-helix and from which a filamentous polymer can be formed (F-actin). The effect of magnetic fields is greater for larger protein aggregates so paracrystals of F-actin were also studied. We explored the feasibility of producing highly orientated samples of F-actin in an appropriate hydrated environment that was suitable for high-resolution structural analysis, which to date has only been studied by alignment by shear and controlled evaporation of the solvent [16].

2. MATERIALS AND METHODS

2.1. *Preparation of actin*

Actin was prepared from acetone powders of rabbit skeletal muscle [17]. On SDS gels the protein ran as a single band of M_r ~42000. Heavily loaded gels showed that the actin was quite free from other thin filament proteins. G-Actin at about 2 mg/ml was dialysed into 0.2 mM ATP, 0.2 mM DTT, 1.5 mM Tris–HCl (pH 7.0). Before use G-actin was polymerised into F-actin by adding KCl to a final concentration of 0.1 M. F-Actin paracrystals were formed by either adding or dialysing in 25–50 mM $MgCl_2$ [18].

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2.2. *Magnetic birefringence measurements*

Samples undergoing reactions were contained in quartz cells usually with a 1 or 2 mm optical path length. The fresh G-actin solutions were contained in quartz cells with a 3 cm optical path length. These cells were placed in a Bitter type magnet with a maximum field of 13 T. The magnetically induced birefringence Δn (λ = 6328 Å) was measured using a combined photoelastic modulator and compensation technique as in [19].

2.3. *Electron microscopy*

Samples for electron microscopy were removed from the magnetic field, applied to a carbon-coated grid and negatively stained with 1% aqueous uranyl acetate. The specimens were examined either in a JEOL 100 CX or a Philips 200 electron microscope.

3. RESULTS AND DISCUSSION

G-Actin (<2 mg/ml) gave rise to very weak birefringence in magnetic fields up to 12.5 T. However, when G-actin and salt solutions were well mixed and placed in the sample chamber with the magnetic field applied a substantial birefringence developed that increased with time (fig.1). Note that because mixing takes place outside the sample chamber there is a delay of 1–2 min before exposure to the field. The time course for the development of birefringence on addition of KCl approximately follows that expected for the polymerisation of actin. The F-actin was therefore tending to become aligned as it was formed. However, the maximum value of the birefringence that we obtained for F-actin is much less than that reached in conditions of near full alignment as attained, for example, by shear [20]. Because the magnetically induced birefringence is positive and F-actin solutions undergoing shear are also positively birefringence with respect to the shear direction, the filament long axis must orient towards the field direction. This is confirmed in fig.2 which shows that paracrystals align parallel to the field.

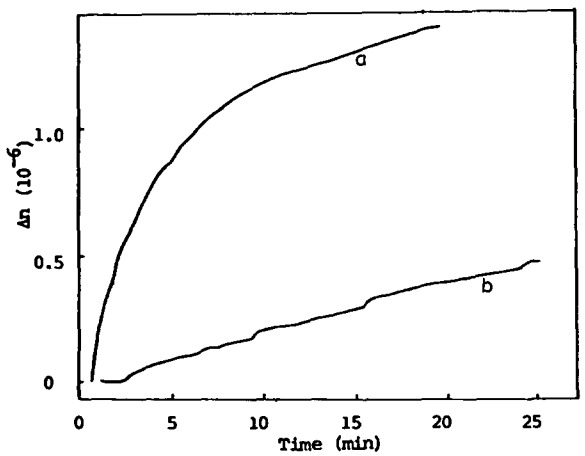

Fig.1. Magnetically induced birefringence, Δn, recorded as G-actin (a) forms paracrystals and (b) polymerises into F-actin in a constant magnetic field of 7.1 T. The G-actin solutions were mixed with salt solutions to a final concentration of (a) 0.5 mg/ml, 35 mM $MgCl_2$; (b) 0.5 mg/ml, 100 mM KCl. Both signals decay slowly and return as the field was withdrawn and reapplied therefore the orientation is magnetically induced and not due to shear alignment as the cells were filled.

Samples undergoing paracrystal formation in high magnesium gave a rapidly rising signal as soon as the birefringence measurements were begun, indicating that paracrystal growth was already well advanced (fig.1). Thereafter the curves increased monotonically towards a limiting value. The most highly orientated sample gave a maximum birefringence of approx. 8×10^{-6} ml/mg which approaches that obtained from F-actin highly aligned by shear [20]. Fig.2 demonstrates that the paracrystals are strongly aligned.

The birefringence arising from F-actin is markedly weaker than that given by F-actin paracrystals (fig.1). With F-actin it is probable that due to polymer entanglement a significant degree of immobilisation set in before the field could be applied and this would result in the orientation being well below the maximum potentially possible. The rapidity of paracrystal formation also restricts the degree of orientation finally reached. To attain maximum magnetic orientation the rate of assembly should be slow enough to allow orientational equilibrium. We therefore reduced the speed of actin polymerisation and paracrystal formation by dialysing the protein against the salt solution overnight in a magnetic field of 7 T given by a superconducting magnet. During these experiments the birefringence could not be monitored. The specimens of F-actin at the low

Fig.2. Optical micrograph taken between crossed-polarisers of a sample of actin paracrystals (0.5 mg/ml, 50 mM KCl, 25 mM $MgCl_2$) formed in a magnetic field of 10 T. The arrow shows the field direction. Magnification × 100.

protein concentration used (<1 mg/ml) were fragile and proved difficult to manipulate. However, we were able to remove F-actin paracrystals from the dialysis bag while taking care to note the direction of the field relative to the sample. These samples, although partly disturbed by manipulation, were observed in the optical microscope to consist of highly birefringent bundles of paracrystals that had aligned parallel to the field (fig.2). Small fragments of these bundles examined in the electron microscope showed that they were composed of the usual Hanson type magnesium paracrystals [21] where the filaments appear parallel and are several layers thick. Thus one can expect that highly orientated paracrystals suitable for structural studies with X-ray diffraction will be produced. It is more doubtful that this will be possible with F-actin but the results are encouraging and experiments should be tried at higher protein concentration. These initial results suggest that as well as improving orientation slow formation in the magnetic field might increase the order and size of actin paracrystals.

The three-dimensional structure of actin monomer at 6 Å resolution is known from X-ray diffraction analysis of crystals of actin complexed with DNase I. The actin subunit contains two domains [22]. Although poorly resolved at 6 Å each appears to consist of α-helices on the outer surface surrounding centrally located β-pleated sheet and appears to have its helices orientated in the same direction. A relatively low resolution picture of the actin monomer has also been obtained from electron microscope studies of F-actin paracrystals and F-actin tropomyosin paracrystals [23–25] as well as polymorphic aggregates of actin induced by gadolinium [26–28]. However, the orientation and detailed interactions of actin subunits in filaments have not been unambiguously determined (review [29]). Our data place some restriction on how the subunit may be incorporated into the filament. Actin contains about 30% α-helix and about 26% β-pleated sheet [30] with the amino acid sequence consisting of less than 10% aromatic amino acids [31]. The diamagnetic anisotropy of β-pleated sheets is only about 1/4 of that of α-helices [12]. The aromatic residues are potentially more anisotropic than the α-helices but are unlikely to have a preferred orientation within a subunit. It has been pointed out that whenever there is a significant proportion of preferentially aligned α-helix in a protein it will almost certainly dominate the diamagnetic properties [12]. Thus, as both α-helices and F-actin orient parallel to the field it

suggests that the α-helices in each subunit are approximately aligned along the actin filament.

If the α-helices are approximately aligned along the actin filament their exact position on the surface of each actin subunit might have functional significance. The other thin filament protein tropomyosin, which moves in relation to actin during muscle contraction [32–34], is a coiled-coil α-helix which may interact to provide specific coiled-coil, protein-protein bonds between actin and tropomyosin.

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