Hydrodynamic modelling of the solution conformation of 10 S myosin

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Received: 10 May 1995
Accepted: 19 June 1995

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Abstract A great deal of work has been done to further the understanding of the solution conformation of the 6 S myosin monomer. Less extensively studied is the 10 S conformer in which part of the tail is observed to be attached to the heads. In this paper existing hydrodynamic data are further interpreted. A model for 10 S myosin is proposed which is more compact than previously reported structures.

Key words hydrodynamic bead modelling – 10 S conformation – myosin

Introduction

The solution conformations of myosin [1–5] and its subfragments [6, 7] have been widely studied and the data obtained have been analysed at a number of resolutions to further the understanding of the role of this molecule in the function of muscle. Specifically, whole myosin has been modelled hydrodynamically in two distinct ways. Its representation as a low resolution general triaxial ellipsoid [8, 9] results in an axial ratio \((a/b, b/c) = (80, 1)\). But this strategy is insufficient to account for the mass distribution and inevitable flexibility of the molecule. The controversy surrounding myosin flexibility has been the subject of extensive work (see [10] and references cited therein) but it is almost certain that there are two points of flexibility within whole myosin: at the joint between the heads (S1 subfragments) and the tail (S2 and LMM) [10] and between the S2 and LMM sections of the tail itself [11, 12].

Myosin exists in a number of forms within the body. It is found in smooth muscle, in striated muscle and also in non-muscle cells. Monomer 6 S myosin self-associates to form myosin filaments. A 10 S conformer of myosin has also been observed both in smooth [13] and striated [4] muscle and in some non-muscle cells. In this inert conformer a region of the tail appears (under the electron microscope) to be attached to the neck region. The role of these conformers is uncertain, their presence in vivo has yet to be confirmed. However the solution conformation has been studied with small-angle x-ray scattering [3, 5]. The apparent radius of gyration of cross-section was 17 Å for the 6 S and 32 Å for the 10 S myosin. Sphere models were constructed for both conformations and the wider angle scattering curves generated for these objects using Debye equations were compared with experimental data. The authors found it necessary to model 6 S myosin with an unexpectedly small angle between the S1 subfragment heads. They also observed that their 10 S model with its openly-looped tail gave a comparatively poor fit to the scattering data.

In this present study sphere (or bead) models for 6 S and 10 S myosin are presented based upon the emulation of hydrodynamic data. In one model for the intact molecule the tail is represented, in common with Faruqi et al. (1991) by spheres of 20 Å diameter. But unlike the Debye sphere model reported by these authors, the heads of the hydrodynamic models reported here have been generated with an algorithm which facilitates the direct transformation of atomic coordinate data into bead coordinates [14]. With the release of the coordinates for the α-carbon backbone of the S1 fragment [15] it is now possible to generate models confident of the limits imposed by steric hindrance in, for example, bringing the heads together in 6 S myosin.
Hydrodynamic bead modelling

The modelling of biological macromolecules with multiparticle assemblies is well documented and will not be explored at length in this paper. For a good review of the theory upon which hydrodynamic modelling is based see [16]. The program TRV written by Garcia de la Torre [17] uses a modified Burgers–Oseen tensor to generate an array of reduced hydrodynamic parameters from the Cartesian coordinates and radii of the bead assembly. An updated version of this software is also available (HYDRO [18]) which, amongst other improvements over TRV, converts the reduced parameters to experimentally relevant data. The program used in this study is a version of TRV modified to do just this and to link directly with the model generation algorithm [19]. The hydrodynamic modelling was performed on a Silicon Graphics Instruments Challenge XL mainframe computer at the University of Leicester, UK.

The design of models

The remarkable discrepancies in hydrodynamic data obtained by different laboratories [10, 11] extend beyond the parameters of rotational relaxation times and rod length. A cursory survey of work published in the last four decades reveals a significant variation in molecular weight (M), sedimentation coefficient (s20,w), diffusion coefficient (D), intrinsic viscosity ([η]) and radius of gyration (Rg) for intact 6 S myosin although the data for the S 1 subfragment are less variable [6]. It has been suggested that myosin isolated from the experimental animal might indeed be changing as the animal is inbred over time [20]. The modelling of the 6 S and 10 S conformers reported in this paper aims to reproduce the most recent data which are given in Table 1.

A further limitation to the level of accuracy fundamentally achievable in hydrodynamic modelling is the uncertainty in the level of molecular hydration (δ). Although recently a reliable method for the determination of δ was reported [21] in this case an alternative derivation was possible for the intact molecule, requiring no further experimental work. The ratio of regression coefficients for the concentration dependence of viscosity and sedimentation coefficients (κn and κs respectively) is equal to the ratio of swollen and partial specific volumes (v̅ and v respectively) [22].

\[
\frac{\kappa_n}{\kappa_s} = \frac{\bar{v}_s}{\bar{v} + \delta}.
\]  

From the data in Table 1 this ratio is 1.054 ± 0.141. The hydration can then be calculated from the following relationship, as v̅ for whole myosin is known to be 0.728 ml/g.

\[
\bar{v} = 1 + \frac{\delta}{\bar{v}}.
\]  

As δ cannot be negative, the effective range of hydration is (0 ≤ δ ≤ 0.14) g water/g protein. This is a low level of solvation (an average protein solvation is around 0.25–0.35 g water/g protein). To compensate for any underestimation in hydration the maximum value of δ = 0.14 g/g was used in all modelling presented here. It was assumed that there would be no change in hydration upon change in conformation and that hydration is uniform.

The models were hydrated by uniform expansion [11]. The ratio of hydrated volume to dry volume given in (2) above for δ = 0.14 g/g is 1.19. This ratio is the cube of the expansion factor (1.06). Thus in order to simulate the required hydration a 6% uniform expansion was applied so that all radii were increased by 6% and the coordinates were similarly modified so that the final model was essentially a magnified copy of the anhydrous starting assembly.

An edited version of the Brookhaven PDB [23, 24] file for the atomic coordinates of the α-carbon backbone of myosin subfragment S 1 [15] was submitted to the algorithm AtoB [14] for the generation of a bead assembly representation of the myosin head region. A resolution of 20 Å was adopted in order to reduce the number of constituent beads and accordingly the computational time required. This resolution notionally represents the maximum diameter a bead can have in the model. But in order to simultaneously satisfy the constriction of v̅, molecular weight and the atomic coordinates, some of the spheres in the model are larger than 20 Å in diameter and overlap with each other. In this instance TRV makes a good approximation as the mathematical basis for computing interaction tensors for overlapping beads of non-uniform size is as yet undeveloped.

Two models were constructed for 6 S myosin (Fig. 1)—one (T) generated solely from atomic resolution data, the

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<th>Table 1 Comparison of experimentally determined and hydrodynamically modelled parameters for bead models of 6 S myosin</th>
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<tr>
<td><strong>Parameter</strong></td>
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</tr>
<tr>
<td>M° (kDa)</td>
</tr>
<tr>
<td>v (ml/g)</td>
</tr>
<tr>
<td>s20,w (S)</td>
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<td>k (ml/g)</td>
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<td>[η] (ml/g)</td>
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<td>κ (ml/g)</td>
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¹ [26]; ² [27]; ³ [1]; ⁴ [2].