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So near and yet so far from understanding molecular motors: recollections in honor of John T. Edsall

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Memories of early years in John T. Edsall's laboratory, 1957–1959

I came to John Edsall's laboratory in the fall of 1957, having just completed my Ph.D. in the Physical Chemistry department at Yale University. Although J.G. Kirkwood was ostensibly my thesis advisor at Yale, I had little contact with Kirkwood, who was widely known for his contributions to polymer hydrodynamics, thermodynamics and statistical mechanics. I spent most of my first two years titrating various unrelated proteins in connection with Kirkwood's theories on the ionic interactions of peptides and proteins [10], but during my third and last year at Yale, I had the opportunity to work on the size and shape of myosin by light scattering [5]. At that time, myosin was thought of more as a good model system for helical polypeptides, than as a biologically interesting molecule. Towards the end of my stay at Yale, it became known that trypsin could cleave myosin into a water soluble component, heavy meromyosin (HMM) and a filament forming component, light meromyosin (LMM), but how these subfragments related to the native parent molecule was not known.

Because John Edsall had studied the flow birefringence of 'myosin' (actually actomyosin) at an early stage in his career, a study from which it was concluded that the protein behaved as an asymmetric, rod-like molecule [24], he had a continuing interest in muscle contraction, and suggested I join his laboratory to pursue my interests in muscle proteins. Another postdoctoral fellow in the laboratory, Don Wetlaufer, was following up on the small subunits released by myosin upon exposure to urea, an observation first made by Tsao in 1953. During the first year, I briefly participated in the application of Raman spectroscopy to the study of small molecules with reactive thiol groups [17], but my attention soon returned to the problem of myosin and actin, and I continued the experiments with the meromyosins first begun at Yale.

Around the time that I entered his laboratory, John Edsall became Editor-in-Chief of the *Journal of Biological Chemistry*, a position that obviously took a great deal of time and energy given his desire to elevate the journal to even higher standards of excellence. Despite the burden of editorial duties, Dr Edsall took an active interest in my myosin research, and was always supportive of my efforts. This was particulary important, since Jim Watson and his associates were in the midst of their

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pioneering work on ribosomes, work which required extensive use of the analytical (Model E) ultracentrifuge. Except for the fact that the Model E was under Dr Edsall's jurisdiction, I would have been severely limited in the number of experiments I could execute in the ultracentrifuge, since the Watson team primarily considered me a nuisance and an obstruction to their more important research. They may well have been correct in their opinion, but I was determined to measure the molecular weights of the meromyosins (by the new 'Archibald' method or approach to sedimentation equilibrium), and analyze the sedimenation velocity patterns for the percentage HMM and LMM in the myosin molecule [11]. Dr Edsall very magnanimously declined to include his name in the authorship of that paper, but it is fair to say that it could not have been completed without his help and diplomacy.

Although I spent only two years at the Biological Laboratories in Cambridge, they had a lasting effect on my vision of proteins as highly intricate macromoleules whose properties can be profoundly influenced by small changes in the ionic environment. I think many of the current controversies and problems in the myosin field are due, in part, to a tendency to overlook the dynamic properties of proteins, especially in the case of the motor proteins, which are designed to exist in so many nucleotide states during their working cycle.

The Jimmy Fund decade, 1960–1970

After Harvard University, I moved to the Children's Cancer Research Foundation (the 'Jimmy Fund') and nearby Harvard Medical School, where I continued the work on myosin in the new Structural Biology Laboratory created by Carolyn Cohen and Don Caspar. I had first met Carolyn Cohen in discussion groups on muscle held by Dr Edsall in his office. Carolyn was already very involved in the biological significance of α-helical coiled–coils in the fibrous proteins. By combining additional hydrodynamic data for the meromyosins with optical rotatory dispersion measurements of their α-helical content, and using insights about coiled–coils gained from wide-angle X-ray diffraction paterns [1], it was possible to arrive at a model for the structure of myosin which had a 'double-stranded α-helical core with a globular mass(es) projecting from the rod' [12]. This structure was remarkably similar to the actual structure visualized by electron microscopy some five years later, and communicated by John T. Edsall to the Proceedings of the National Academy of Sciences [23]. By improved techniques in shadow-cast preparations, it was shown that two globular masses (referred to as subfragment-1 or S1) were attached to the rod, confirming that myosin consists primarily of two polypeptide chains.

The remainder of this period was devoted to dissecting myosin in ever increasing detail by means of proteolytic enzymes, and characterizing the functional domains by enzymatic and hydrodynamic methods [13]. The elucidation of these structural elements (e.g. S1, the globular head containing the nucleotide—and actin—binding sites; and S2, the soluble α-helical rod portion of HMM) contributed to the 'tilting cross-bridge' model as a mechanism for producing relative sliding and generating force between the thick and thin filaments [6]. At about this time, transient kinetic analysis of actomyosin had progressed sufficiently to show that the ATPase cycle included dissociated myosin states followed by strongly bound actomyosin states upon product release [16]. This cycle correlated very well with the available structural information and the sliding filament hypothesis. One could argue that the essential features of the contractile cycle were understood at that stage, but there was really no direct experimental evidence that myosin heads actually behaved that way in vitro or in vivo, and no high resolution crystal structures of myosin or actin were available.

The modern era of motility research

After moving our Structural Biology Laboratory to the newly built Rosenstiel Basic Medical Sciences Research Center at Brandeis University in 1972, we were joined by David DeRosier who introduced new methodologies for analyzing complex macromolecular assemblies by electron microscopy. Our research in the 1970s focused, in part, on clarifying the stoichiometry and function of the light chains, those low molecular weight

subunits which were at one time considered to be possible contaminants of the myosin preparation. Initially, it was thought they might be essential for enzymatic activity, since slow and fast myosin isoforms had unique light chains [14], but subsequent experiments showed the light chains could be removed without appreciable loss of activity. Progress in understanding their role was later provided by the publication of the first high resolution atomic structure of the myosin head, S1 [19].

The first successful crystallization of the myosin motor used the proteolytic fragment, S1, prepared by limited papain digestion of chicken pectoralis myosin [13]. The choice of an avian species rather than the rabbit myosin commonly used in most muscle laboratories was a serendipitous decision resulting from our immunological studies. We were interested in generating specific polyclonal antibodies for mapping myosin isoforms in muscle cells, and injecting rabbits with chicken myosin fragments was far more convenient than injecting goats with rabbit myosin subfragments. In hindsight, the choice of an avian tissue was probably advantageous, since the pectoralis major is a much more homogeneous muscle than most mammalian muscles. Ivan Rayment, a crystallographer working with Don Caspar and Don Winkelmann, a postdoctoral fellow in my laboratory, decided they would try to crystallize S1, and much to everyone's surprise they succeeded. After their first exciting announcement [18], it took another ten years of great effort in Rayment's laboratory before the atomic structure was solved. That, plus the earlier solution of the atomic structure of actin [8], ushered in a new era in our understanding of muscle contraction.

Along with advances in structure, the 1980s saw the introduction of in vitro motility assays [22] which made it possible for the first time to actually see actin filaments gliding over myosin heads on a coverslip, and to measure velocity and force at the molecular level [9]. Further development of these technologies culminated in the ability to measure single steps of 5–10 nm and forces of several pN produced by individual myosin molecules [26]. The atomic structure of the myosin head is very compatible with a working stroke of this magnitude, insofar as it consists of a globular domain from which emerges a long α-helix that is stabilized through interactions with two light chains. In addition to finally providing a role for the small subunits [15], this lever-like domain led to the reasonable hypothesis that small changes in the motor domain caused by nucleotide binding, hydrolysis, and release of products could be amplified into movements of 5–10 nm of the lever arm. Support for this model came from crystal structures of various head fragments expressed in *Dictyostelium discoideum* [3] and in the baculovirus/insect cell system [2], which showed that the lever arm could rotate by as much as 70° depending on the state of the nucleotide at the active site. By engineering cysteine residues at specified sites on the light chains [21], it became possible to attach fluorescent and paramagnetic probes, and thereby introduce labeled light chains into muscle fibers. Such spectroscopic approaches made it possible to directly observe changes in orientation of the lever arm during the contractile event, reinforcing the lever-arm hypothesis [20]. Time-resolved X-ray diffraction measurements from single muscle fibers also provided strong in situ support for this model [7]. Thus, there is now a large body of evidence that a tilt of the light chain domain takes place during the working stroke of myosin (for an excellent review see [4]).

But is this the final story? Since it took ~40 years for the sliding filament 'hypothesis' to become a generally accepted 'fact', the molecular basis for movement has a way to go to reach that level of consensus. There are widely respected scientists whose experiments favor a thermal ratchet or Brownian motion type of mechanism in which the myosin molecule can undergo multiple substeps over a long distance per single ATP turnover, i.e. a loose coupling mechanism.[25]. Moreover, the proponents of this view claim that removal of the light chain-binding domain has no effect on the working stroke, and suggest that the light chains act instead as a strain sensor to modulate myosin kinetics. Expert protein biochemistry and advances in biophysics will be needed to resolve these issues. To arrive at a molecular mechanism for movement, high resolution crystallographic studies of the actin-bound state will also be essential. The power stroke only occurs in the attached state, and

until we can see the domain movements in actomyosin we will not understand how the molecular motor works.

As is evident from this very cursory review of the muscle field, these 'recollections' are more about my 'life with myosin' than about my association with John Edsall. And yet the two are interconnected in many ways: in John's laboratory I was encouraged to continue the work on myosin that I had begun at Yale; without this support and enthusiasm, I might well have gone in another direction. He showed by example what 'fine scholarship' meant, and how the investigator must be the most critical judge of his or her work. Most important, he taught me the art of good writing: science writing need not be dull or redundant, but instead can impart information clearly and succinctly. John Edsall has been a wonderful role model for me in my scientific career. I dedicate this article to his memory, grateful for his many contributions both to science, and to making the world a better habitat for all.

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References

[1] Cohen, C., Szent-Gyorgyi, A.G. The α-class of fibrous muscle proteins. Proc. IVth Intern. Cong. Biochem, Vienna 8: 108–118, 1960.

[2] R. Dominguez, Y. Freyzon, K.M. Trybus, C. Cohen, Crystal structure of a vertebrate smooth muscle myosin motor domain and its complex with the essential light chain: visualization of the pre-power stroke state, Cell 94 (1998) 559–571.

[3] A.J. Fisher, C.A. Smith, J.B. Thoden, et al., X-ray structures of the myosin motor domain of *Dictyostelium discoideum* complexed with MgADP·BeF_x and MgADP·AlF_4^-, Biochemistry 34 (1995) 8960–8972.

[4] M.A. Geeves, K.C. Holmes, Structural mechanism of muscle contraction, Annu. Rev. Biochem. 68 (1999) 687–728.

[5] A.M. Holtzer, S. Lowey, On the molecular weight, size and shape of the myosin molecule, J. Am. Chem. Soc. 81 (1956) 5954.

[6] H.E. Huxley, The mechanism of muscular contraction, Science 164 (1969) 1356–1366.

[7] M. Irving, V. Lombardi, G. Piazzesi, M.A. Ferenczi, Myosin head movements are synchronous with the elementary force-generating process in muscle, Nature 357 (1992) 156–158.

[8] W. Kabsch, H.G. Mannherz, D. Suck, E.F. Pai, K.C. Holmes, Atomic structure of the actin:Dnase 1 complex, Nature 347 (1990) 37–44.

[9] A. Kishino, T. Yanagida, Force measurements by micromanipulation of a single actin filament by glass needles, Nature 334 (1988) 74–76.

[10] S. Lowey, Acid-base equilibrium in solutions of isoionic conalbumin, Arch. Biochem. Biophys. 64 (1956) 111–114.

[11] S. Lowey, A.M. Holtzer, The homogeneity and molecular weights of the meromyosins and their relative proportions in myosin, Biochim. Biophys. Acta 34 (1959) 470–484.

[12] S. Lowey, C. Cohen, Studies on the structure of myosin, J. Mol. Biol. 4 (1962) 293–308.

[13] S. Lowey, H.S. Slayter, A.G. Weeds, H. Baker, Substructure of the myosin molecule I. Subfragments of myosin by enzymic degradation, J. Mol. Biol. 42 (1969) 1–29.

[14] S. Lowey, D. Risby, Light chains from fast and slow muscle myosins, Nature 234 (1971) 81–85.

[15] S. Lowey, G.S. Waller, K.M. Trybus, Skeletal muscle light chains are essential for physiological speeds of shortening, Nature 365 (1993) 454–456.

[16] R.W. Lymn, E.W. Taylor, Mechanism of adenosine triphosphate hydrolysis by actomyosin, Biochemistry 10 (1971) 4617–4624.

[17] R.B. Martin, S. Lowey, E.L. Elson, J.T. Edsall, Hydrolysis of 2-methyl-delta-thiazoline and its formation from *N*-acetyl-beta-mercaptoethylamine. Observations on an N–S acyl shift, J. Am. Chem. Soc. 81 (1959) 5089–5095.

[18] I. Rayment, D.A. Winkelmann, Crystallization of myosin subfragment 1, Proc. Natl. Acad. Sci. USA 81 (1984) 4378–4380.

[19] I. Rayment, W. Rypniewski, K. Schmidt-Base, et al., Three-dimensional structure of myosin subfragment-1: a molecular motor, Science 162 (1993) 50–58.

[20] C. Sabido-David, S.C. Hopkins, L.D. Saraswat, S. Lowey, Y.E. Goldman, M. Irving, Orientation changes of fluorescent probes at five sites on the myosin regulatory light chain during contraction of single skeletal muscle fibres, J. Mol. Biol. 279 (1998) 387–402.

[21] L.D. Saraswat, S. Lowey, Engineered cysteine mutants of myosin light chain 2: fluorescent analogues for structural studies, J. Biol. Chem. 266 (1991) 19777–19785.

[22] M.P. Sheetz, J.A. Spudich, Movement of myosin-coated fluorescent beads on actin cables in vitro, Nature 303 (1983) 31–35.
[23] H.S. Slayter, S. Lowey, Substructure of the myosin molecule as visualized by electron microscopy, Proc. Natl. Acad. Sci. USA 58 (1967) 1611–1618.
[24] A.L. Von Muralt, J.T. Edsall, Double refraction of myosin in flowing solutions, J. Biol. Chem. 89 (1930) 315–350.
[25] T. Yanagida, K. Kitamura, H. Tanaka, A.H. Iwane, S. Esaki, Single molecule analysis of the actomyosin motor, Cur. Opin. Cell. Biol. 12 (2000) 20–25.
[26] J.T. Finer, R.M. Simmons, J.A. Spudich, Single myosin molecule mechanics: piconewton forces and nanometer steps, Nature 368 (1994) 113–119.