material. If it exists, the concentrations of the smaller particles must be very low. Although it is possible to model the fusion of myosin octamers into minifilaments, and perhaps even tetramers into octamers, we have so far no direct way to monitor such transitions except for observing their end products. Consequently, we cannot properly assess the function of myosin monomers and dimers in such reactions.

That dimers might be involved in the initial assembly reactions of myosin is indicated by the presence of 10S species in ATP titrations of the 18S and 22S particles. The 10S material appears to be in equilibrium with the monomeric 6S form of myosin. We assume that the 10S species correspond to the 10S or 11S parallel myosin dimer identified in a separate work.[1] Future work will be directed at testing the importance of the small assemblies of myosin in the formation of myosin minifilaments.

We are grateful to Dr. H. Eisenberg for making his laboratory in the Polymer Department at the Weizmann Institute of Science available to us for light scattering experiments and for his hospitality and help to E. Reisler.

This work was supported by U.S. Public Health grant AM 22031.

Received for publication 24 May 1985 and in revised form June 1985.

REFERENCES

Alexandrowicz, Z., and E. Daniel. 1963. Sedimentation and diffusion of polyelectrolytes. Part I. Theoretical Description. *Biopolymers.* 1:447–471.

Davis, J. S. 1981*a*. The influence of pressure on the self-assembly of the thick filament from the myosin of vertebrate skeletal muscle. *Biochem. J.* 197:301–308.

Davis, J. S. 1981*b*. Pressure jump studies on the length-regulation kinetics of the self-assembly of myosin from vertebrate skeletal muscle into thick filaments. *Biochem. J.* 197:309–314.

Davis, J. S., J. Buck, and E. P. Greene. 1982. The myosin dimer: an intermediate in the self-assembly of the thick filament of vertebrate skeletal muscle. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 140:293–297.

Eisenberg, H. 1971. Light scattering and some aspects of small angle x-ray scattering. *In* Procedures in Nuclear Acid Research. G. L. Cantoni and D. R. Davies, editors. Harper and Row Publishers, New York. 2:137–175.

Eisenberg, H., and E. Reisler. 1971. Angular dependence of scattered light, rotary frictional coefficients, and distribution of sizes of associated oligomers in solutions of bovine liver glutamate dehydrogenase. *Biopolymers.* 10:2363–2376.

Elovson, J., J. C. Jacobs, V. N. Schumaker, and D. J. Puppione. 1985. Molecular weight of apoprotein B obtained from human low-density lipoprotein (apoprotein B-PI) and from rat very low density lipoprotein (apoprotein B-PIII). *Biochemistry.* 24:1569–1578.

Godfrey, J. E., and W. F. Harrington. 1970. Self association in the myosin system at high ionic strength. I. Sensitivity of the interaction to pH and ionic environment. *Biochemistry.* 9:886–893.

Harrington, W. F., and M. Burke. 1972. Geometry of the myosin dimer in high salt media. I. Association behavior of rod segments from myosin. *Biochemistry.* 11:1448–1455.

Harrington, W. F., and S. Himmelfarb. 1972. Effect of Adenosine di- and triphosphates on the stability of synthetic myosin filaments. *Biochemistry.* 11:2945–2952.

Harrington, W. F., and M. E. Rodgers. 1984. Myosin. *Annu. Rev. Biochem.* 53:35–73.

Higuchi, H., and Ishiwata, S. 1985. Disassembly kinetics of thick filaments in rabbit skeletal muscle fibers. *Biophys. J.* 47:267–275.

Huxley, H. E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* 7:281–308.

Ishiwata, S. 1981. Melting from both ends of an A-band in myofibrils. Observation with a phase contrast miscroscope. *J. Biochem.* 89:1647–1650.

Jolly, D., and H. Eisenberg. 1976. Photon correlation spectroscopy, total intensity light scattering with laser radiation and hydrodynamic studies of a well fractionated DNA sample. *Biopolymers.* 15:61–95.

Josephs, R., and W. F. Harrington. 1966. Studies on the formation and physical properties of synthetic myosin filaments. *Biochemistry.* 5:3474–3487.

Maw, M. C., and A. J. Rowe. 1980. Fraying of A-filaments into three subfilaments. *Nature (Lond.).* 286:412–414.

McLachlan, A. D., and J. Karn. 1982. Periodic charge distributions in the myosin rod amino acid sequence match cross-bridge spacing in muscle. *Nature (Lond.).* 299:226.

McLachlan, A. D., and J. Karn. 1983. Periodic features in the amino acid sequence of nematode myosin rod. *J. Mol. Biol.* 164:605–626.

Niederman, R., and L. K. Peters. 1982. Native bare zone assemblage nucleates myosin filament assembly. *J. Mol. Biol.* 161:505–517.

Oriol-Audit, C., J. A. Lake, and E. Reisler. 1981. Structural changes in synthetic myosin minifilaments and their dissociation by adenosine triphosphate and pyrophosphate. *Biochemistry.* 20:679–686.

Pepe, F. A. 1982. The structure of vertebrate skeletal-muscle myosin filaments. *In* Cell and Muscle Motility. R. M. Dowben and J. W. Shay, editors. Plenum Publishing Corp., New York. 2:141–177.

Pepe, F. A. 1983. Macromolecular assembly of myosin. *In* Muscle and Nonmuscle Motility. A. Stracher, editor. Academic Press, Inc., New York. 1:105–149.

Reisler, E., M. Burke, R. Josephs, and W. F. Harrington. 1973. Cross-linking of myosin and myosin filaments. *J. Mechanochem. Cell Motility.* 2:163–179.

Reisler, E., C. Smith, and G., Seegan. 1980. Myosin minifilaments. *J. Mol. Biol.* 143:129–145.

Reisler, E., P. Cheung, C. Oriol-Audit, and J. A. Lake. 1982. Growth of synthetic myosin filaments from myosin minifilaments. *Biochemistry.* 21:701–707.

Scheraga, H. A., and L. Mandelkarn. 1953. Consideration of the hydrodynamic properties of proteins. *J. Amer. Chem. Soc.* 75:179–184.

Trinick, J., and J. Cooper. 1980. Sequential disassembly of vertebrate muscle thick filament. *J. Mol. Biol.* 141:315–321.

Zimm, B. H. 1948. Apparatus and methods for measurement and interpretation of the angular variation of light scattering; preliminary results on polystyrene solutions. *J. Chem. Phys.* 16:1099–1116.

DISCUSSION

Discussion Chairman: Thomas D. Pollard
Scribes: Gillian Henry, John Smuda, and Ayuko Yotsukura

GERGELY: Why were the endpoints different when you added KCl to the 18S and 22S particles?

REISLER: The end product is controlled by the amount of KCl. By

addition of measured amounts of KCl, the 18S can be transformed to the 22S and subsequently to minifilaments. The stable end product is minifilaments.

VIBERT: Are there any metastable oligomers other than the tetramers, the octamers, and the minifilaments?

REISLER: As far as we can see, these are the only ones. However, we cannot preclude the existence of others; they might exist in concentrations too low to detect.

VIBERT: If these are "precursors" of the native filament, how do you relate the packing geometry of the minifilaments to the native filament?

REISLER: So far, this type of modelling agrees best with Pepe's models (see references).

VIBERT: Given that the native filament probably has three-fold rotational symmetry, is there a simple way of picturing how the minifilament relates to the native filament?

REISLER: You can envisage the minifilament as one-third of the central portion of the native filament. Splaying experiments, of the type done by Rowe and subsequently by Pepe and by ourselves, using solvents that produce minifilaments, reveal three substructures, held together only in the central region. Each one of those substructures might correspond to a minifilament. Assuming a 16-mer with eight monomers pointing in each direction, there would be 24 monomers in cross-section, one-third of such a structure. The pyrophosphate experiments were done because myosin myofibrils were shown to be dissociated by pyrophosphate treatment. We started by dissociating native filaments with pyrophosphate and ended up with minifilaments.

POLLARD: I don't understand how you can use these minifilaments to make the bare zone of a conventional filament.

REISLER: A fusion of three of the 16-mers would correspond to the central region. The transition from the octamer to the minifilament is a fusion process, but going from the minifilament to the full-size filament requires growth, a process that requires more time. We cannot follow the individual steps, and we can't tell, at this stage, whether the thickening of the filament is achieved by addition of dimers or simply by fusion. Perhaps fluorescence resonance energy transfer experiments, as performed by Anuradha Saad, will be helpful in distinguishing between fusion and simple growth.

POLLARD: If these are parts of the bare zone of a thick filament, then the heads ought to be on one side, whereas your electron micrographs seem to show the heads pointing in all directions. If this is going to be a third of the thick filament bare zone, you could imagine that it would be impossible to have the heads on the inside.

REISLER: I don't really know how much the orientation of heads in the electron micrograph corresponds to orientation in the preparation, because we normally cross-link our specimen. Depending on the extent of cross-linking, the heads are sometimes no longer visible.

STEWART: One of the ideas that Kensler presents (this volume) is that the heads of vertebrate myosin filaments (such as these) are perturbed in a number of ways. One way is their arrangement into three subfilaments within the filament. The heads have to be distorted to get to the outside. I think that this distortion of the heads is consistent with the idea that minifilaments form part of the bare zone. This could be one, although not the only, explanation of the distortion of the heads that we see.

REISLER: I agree.

VIBERT: My sense of your minifilaments is that the length of the bare zone is almost twice the length of the myosin rod minus 43 nm, whereas in native filaments, the length of the bare zone is of the order of one myosin rod, ~ 1,500–1,600 A.

REISLER: I don't have an answer to that.

SAAD: Have you made minifilaments of myosin from other sources, for example, *Nematoda*? It has been shown that there are two genetically different forms of nematode myosin. One forms the bipolar bare zone and the other, the more distal parts of the filaments.

REISLER: No, we have not.

SAAD: In an *in vitro* assembly system, your filaments are very stable entities. How do you visualize the assembly occurring? If you start off with monomers and dimers, why does the process stop at minifilaments and not go on to form filaments?

REISLER: The minifilaments are stable only under very artificial ionic strength and solvent conditions. These are not what we normally have when we monitor filament assembly or those that exist in the cell. Pyrophosphate minifilaments are much more easily perturbed. They can be isolated under stable conditions, but further growth can easily be induced.

SAAD: Do you think a *Nematode*-like system can exist in vertebrates?

REISLER: There is no way of telling. We do not see any difference between myosin that forms minifilaments and myosin that does not, but we have not looked at the sequence of these myosins or at microheterogeneity.

SAAD: C-protein and M-protein bind only to the center. It would be interesting to see if there are two different types of myosin.

POLLARD: There is some information about two different types of myosin in chicken skeletal muscle, based on unpublished antibody work done by Albert Wong in my lab. He made antibodies against human platelet myosin and a subset of these antibodies reacted with all species tested. When he stained skeletal muscle with these antibodies, he found that they only stained the center of the thick filament.

REISLER: Perhaps we should add the report of Niederman and Peters in which they isolate what they call "bare zone assemblages". This could be an indication of two types of myosin.

LEVINE: Returning to *Nematode* myosin, H. Epstein has some pictures showing assembly of full thick filaments of both myosin types.

REISLER: I had the impression that both chicken isozymes form full thick filaments.