NEWS AND VIEWS

Links Between Chains

It took probably a century of man years to decide whether myosin was two or three stranded. No sooner had the dust of that wrangle settled than another argument was on: low-molecular weight proteins were apparently shed by the myosin molecule, under conditions that led always to denaturation, or at least to loss of ATPase activity. Were these "light chains" real components of the functional molecule, or merely bound impurities? How many species are there, and in what proportion are they present on the myosin? Are they involved in the ATPase function? Does their heterogeneity perhaps arise from different populations of fibres? It may be hoped that two meticulous studies in this edition of *Nature* will help shake off the incubus of numerological uncertainty that has so long dogged the field.

Lowey and Risby (page 81) have addressed themselves to the general problem of the estimation of protein components in mixtures by gel electrophoresis. The light and heavy chains cannot be compared on a single polyacrylamide gel, because the light chains diffuse too rapidly in a gel of low concentration, whereas the heavy chains will not penetrate a gel of high concentration. Comparisons have therefore to be made between the components in separate gels, and a protein of intermediate molecular weight, tropomyosin, is added to the mixture to provide a common concentration standard for both gels. The linearity of integrated staining intensity with the quantity of protein applied to the gels was demonstrated, and the same relative concentrations of components were obtained with the use of two different protein stains. Moreover, when separated light and heavy chains at defined concentrations, based on nitrogen determination, were run on gels, the staining intensities were found to reflect faithfully the relative concentrations of the different components—a result which, not necessarily general, should give comfort to workers in other branches of protein chemistry. Using molecular weights based on mobility in gels containing the detergent SDS, the stoichiometry of all the components could then be evaluated.

The upshot is that in three fast-muscle preparations, from rabbit and chicken, each molecule of myosin gives rise to four light chains, two of a species of 18,000 molecular weight and two others of 16,000 and 20,000 respectively. It is the first kind that are liberated by treatment with a thiol-specific reagent (Ellman reagent), and their loss is not accompanied by any major change in any measured properties of the myosin, ATPase included. Slow muscles give a quite different pattern: two species of light chain are resolved, which do not correspond to any of the above components, have molecular weights estimated at 20,000 and 27,000, and are present in a ratio of two moles of each per mole of myosin. The results in terms of the number of components, and their differences, as between skeletal and cardiac myosins, are, it is agreeable to report, in substantial agreement with a recent parallel study by Sarkar, Sreter and Gergely (*Proc. US Nat. Acad. Sci.*, **68**, 946; 1971).

In red muscle it looks as though both types of myosin may be present, with five light chain components clearly discernible. The identical patterns from the muscles of two animals are particularly satisfactory features of these results, encouraging, as they do, the confidence that the stoichiometry is correct, and the heterogeneity not adventitious. Moreover, the difference between the light chains from fast and slow muscles, allied to their apparent inseparability from the myosin ATPase activity, raises the alluring possibility that they in fact regulate this function.

In the companion article, Weeds and Pope (page 85) examine the chemical relation between the various light chains. The identifying feature which they have selected is the sequence of the proteolytic fragments containing thiol groups, because these can be labelled with an isotopic alkylating agent and can thus readily be identified after electrophoretic separation of a chymotryptic digest. By this technique Weeds previously demonstrated that the inessential light chain component released by Ellman reagent contains two unique thiol peptide sequences, whereas the remaining light chains possess only one between them. Working this time with prime beef, Weeds and Pope find that cardiac (slow muscle) myosin light chains give rise in all to three different thiol peptides, one of which, a fragment of thirteen residues, is identical to the single thiol sequence present in the ATPase-associated light chains of skeletal muscle myosin.

Now the cardiac myosin light chain preparation contains two different components. Complete separation of these has not been achieved, but it seems certain that one (the larger, with molecular weight 27,000) is responsible for all three thiol peptides, whereas the smaller, of 20,000 molecular weight, most probably possesses no thiols. Sheep heart muscle myosin contains similar light chains, which give rise to the selfsame thiol sequences as those of beef heart myosin. Rabbit cardiac myosin is evidently again similar. The significance of the long common thiol peptide segment between light chains from cardiac and skeletal muscle is not obvious, for in other respects, including the molecular weight, the chains are distinctly different.

Weeds and Pope add that their unpublished results on fast-twitch and slow-twitch muscles of the cat show light chain patterns which are respectively similar to skeletal and cardiac muscle myosin. These results point once more to an enzymatic regulatory function for at least some of the light chains. The heterogeneity remains a curious feature; it may be noted that a current report (Hale and Beecher, *FEBS Lett.*, **18**, 245; 1971) has started once more the hare of heterogeneity of the heavy chains. Gel electrophoresis of the denatured chains in concentrated urea solution in the hands of these workers produces two components in equal concentration. They have done their best to control the rather extensive possibilities of chemical or physical artefacts, but workers in the field may wish to reserve judgment.

If these results are taken at face value, however, they raise now the question of whether the two components represent different halves of all myosin molecules, or different myosin populations. Much work remains to be done on myosin, but there should be no mistake about the importance of establishing the composition of the molecule in definitive terms.