APPENDIX

Calculation of the Charge of the Phosphorylatable Light-Chain Components of Myosins from Different Muscle Types

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Electrophoretic mobility depends solely on the effective radius and charge of a protein when conditions of electrophoresis are held constant, thereby eliminating (i) the direct effect of solvent on the protein, (ii) the electrophoretic and electrokinetic effect of solvent movement, and (iii) the retarding effect of gel concentration. Thus mobility (m) is directly proportional to charge (c) for a protein under constant conditions and the ratio of mobilities of proteins of equal effective radius is equal to the ratio of their respective charges.

$$m_A/m_B = c_A/c_B \quad (1)$$

Similarly introducing changes in the charge (Δc) of a protein causes corresponding changes in mobility (Δm) if the modification does not affect the conformation significantly (i.e. alter the effective radius).

$$\Delta m/m = \Delta c/c \quad (2)$$

Phosphorylation of the 18 500-dalton light chain of skeletal-muscle myosin introduces a single phosphate group (Perrie *et al.*, 1973) which at pH 8.6 increases the net negative charge by 2. By assuming the effective radius is unaltered, the charge on this light chain can be calculated by substituting the measured mobilities before and after phosphorylation, and $\Delta c = -2$ in eqn. (2). If red- and cardiac-muscle myosin 19000-dalton light chains and their modified

forms are of very similar effective radius, their net charges can be calculated by substituting their measured mobilities, and that of the 18 500-dalton light chain and its calculated net charge, in eqn. (1).

Samples of both unphosphorylated and phosphorylated myosins obtained from different muscles were subjected to electrophoresis simultaneously in a single polyacrylamide gel, in 8M-urea at pH 8.6 (see the Methods section) and the migration of each band was measured at least three times. This was repeated several (three or more) times for each of a number of preparations of each myosin and calculations were performed as described above. Measurements of the 19000-dalton cardiac-muscle components were indistinguishable from those of the red-muscle 19000-dalton components.

The net negative charge at pH 8.6 calculated for the 18 500-dalton component was -14.8 , that of the phosphorylated form therefore being -16.8 . Both of these forms occasionally showed minor satellites (calculated charge -15.8 and -18.0). Similarly, the net negative charges calculated for the 19000-dalton component, its phosphorylated form and their respective 'satellites' were -16.8 and -18.7 , -17.8 and -19.5 .

Reference

Perrie, W. T., Smillie, L. B. & Perry, S. V. (1973) *Biochem. J.* **135**, 151–164