Four cross-bridge strands and high paramyosin content in the myosin filaments of honey bee flight muscles

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Summary. Measurements of the mass ratio of myosin to paramyosin of myofibrils of honey bee flight muscles on sodium dodecyl sulphate-polyacrylamide gels yielded a paramyosin content of 24 % of the myosin filament mass. Based on the myosin to actin mass ratio of 2.3, and 3 actin filaments per myosin filament and per half sarcomere, it could be calculated that there were 3.8 myosin molecules repeating regularly at intervals of 14.4 nm along the myosin filament. In spite of the high paramyosin content the diameter of the myosin filaments is 19–20 nm, as in other insect flight muscles.
Key words. Insect muscle; myosin filaments; cross-bridges; paramyosin.

Paramyosin has been found in the muscles of many invertebrates[1]. It is believed to form the backbone of the myosin-containing filaments. The thick filaments of insect flight muscles seem to contain this protein in amounts of 2.5 % to 11.5 %[2, 3, 11]. In spite of different paramyosin contents the diameters of the thick filaments of these muscles are about the same (19–20 nm). The present study will show that the content of paramyosin in the myosin filaments of the flight muscles of the honey bee is larger than that in other insect flight muscles. Myosin content and filament diameter, however, are not different. This suggests a closer packing of the paramyosin molecules in the filament core.

The thick filaments of honey bee flight muscles are myosin filaments with a solid core[3, 4]. Transverse sections (fig. 1 a) show filaments with a diameter of about 20 nm. Electron-dense spots are distributed all over the cross sections of the filaments. Unstructured centers with slightly reduced density compared to the wall, as often seen in cross sections of solid filaments of the fleshfly (fig. 1 d), have not been observed in bee filaments.

The lengths of the sarcomeres varied between 2.0 µm and 2.8 µm within the flight muscle fibers of 4 bees (± 0.2 µm within one fiber) without any indication of different shortening states. As in other insect flight muscles[3] there are three actin filaments per half sarcomere and per

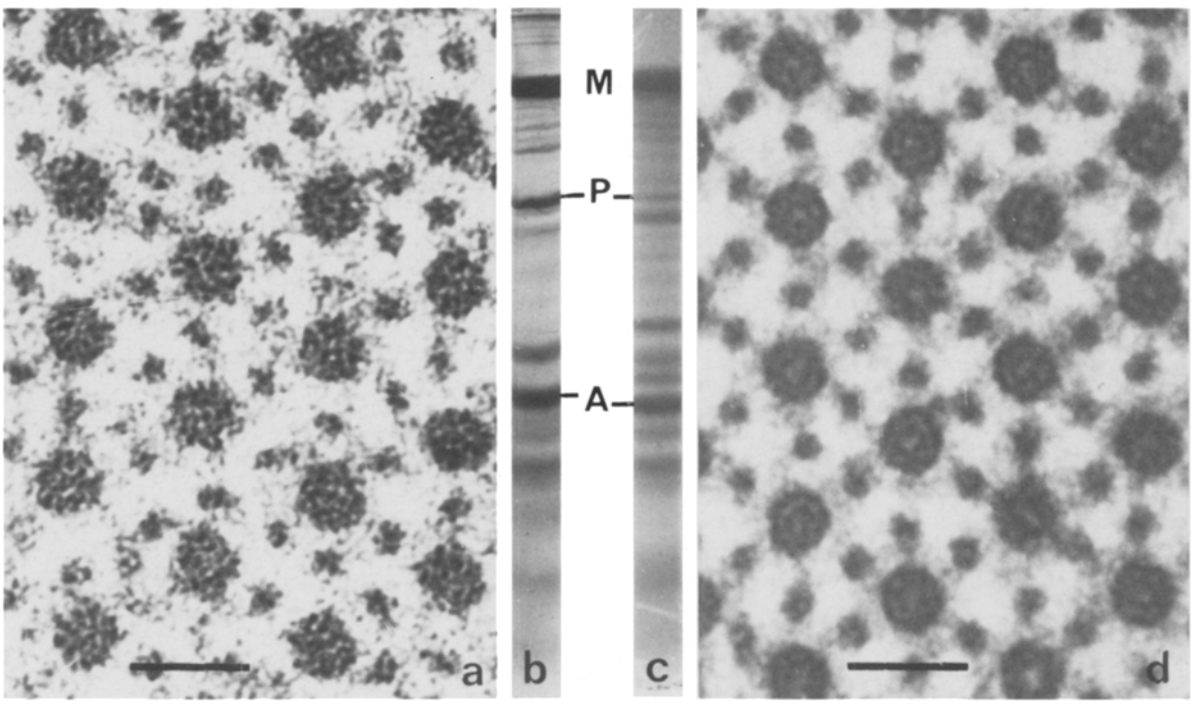

Figure 1. *a* and *d* Cross section (120 nm thick) through sarcomeres of a honey bee (*a*) and a fleshfly, *Phormia terrae-novae*, (*d*) flight muscle (Jeol EM 200 A, 200 kV). Each actin filament is located exactly between two myosin filaments. The myosin filaments of the honey bee show dotlike substructural elements all over their cross section, whereas those of the fleshfly seem to have dot-free centers. The muscle fibers were fixed in situ at resting length. The cytological treatment was done according to Ashton and Pepe[12]. Magnification × 320,000 (bars 50 nm).
b and *c* 5 % Sodium dodecyl sulfate-polyacrylamide gels loaded with 24 µg of myofibrillar proteins of honey bee (*b*) respectively fleshfly (*c*) flight muscles. They were stained with Coomassie Brilliant Blue G 250. Note the different widths of the paramyosin bands. A = actin, P = paramyosin, M = myosin.

myosin filament ($\beta = 3$). The ratio of the lengths of two (one of each half sarcomere) actin filaments (1_a) and one myosin filament (1_m) could be taken to be approximately 1 ($1_a : 1_m = 1$)[6]. The widths of the Z-discs (1_o) and the pseudo-H zones (1_b) were 0.1 μm and 0.15 μm, respectively[6]. These values, together with the molecular weights of actin (m_a = 42 kDa) and the heavy chain of myosin (m_{HC}) = 200 kDa), and the mass ratio of myosin-HC to actin (α) on SDS gels were used for the calculation of the number (N) of myosin molecules per crown (number of myosin molecules repeating regularly at intervals of 14.4 nm (p_m) along the length of the myosin filament)[5]:

$$N = \alpha \times \beta \times \frac{m_a \times p_m}{2\, m_{HC} \times p_a} \times \frac{1_a + 1_o}{1_m - 1_b}$$

The subunit repeat of the actin filaments (p_a) was taken to be 2.74 nm[5]. This is half of the distance of the actin molecules along one actin helix of a thin filament. The factor arises because there are two actin helices forming one thin filament (double helix)[5].

To quantify the myosin and paramyosin content of the filaments, myofibrils from fresh flight muscles of the honey bee *(Apis mellifica)* were purified and prepared for gel electrophoresis according to Tregear and Squire[5]. Three preparations of myofibrils were used. 5 % gels were run, stained (fig. 1 b) and evaluated as described earlier[3]. Individual calibration curves were used for actin (from rabbit skeletal muscle), myosin and paramyosin (both from bee flight muscle) to compensate for the different staining behavior of these proteins[3,5] with Coomassie Brilliant Blue G 250 (Serva, Heidelberg).

In figure 2 (upper graph) the peak areas of myosin bands of individual gels were plotted versus the peak areas of the corresponding actin bands after correction for the different staining behavior of the proteins (see above). The slope of the regression line is 2.31. On this basis it can be calculated that there are 4.2 actin molecules per myosin molecule (= 2 myosin heavy chains); N would be 3.82. This value is close to 4 myosin molecules per crown and corresponds to the finding of 4 helical cross-bridge strands, as proposed for the structure of the myosin filaments of other insect flight muscles[3,7].

The lower graph of figure 2 gives the ratio of paramyosin to myosin, assuming that paramyosin stains 19 % better than myosin heavy chain (the differences in the staining behavior are not shown here). The slope of the regression line is 3.01, giving a molar ratio of myosin to paramyosin (mol. wt 105 000) of about 1.6. This suggests that the thick filaments of the honey bee flight muscles contain about 24 % of paramyosin if other proteins (like M-line or connecting proteins) are not considered. This is about twice as much paramyosin as the 9–11.5 % which have been found in the solid filaments of other insect flight muscles[2,3,11].

Studies of cross sections of myosin filaments of insect flight muscles suggest that the myosin molecules of a

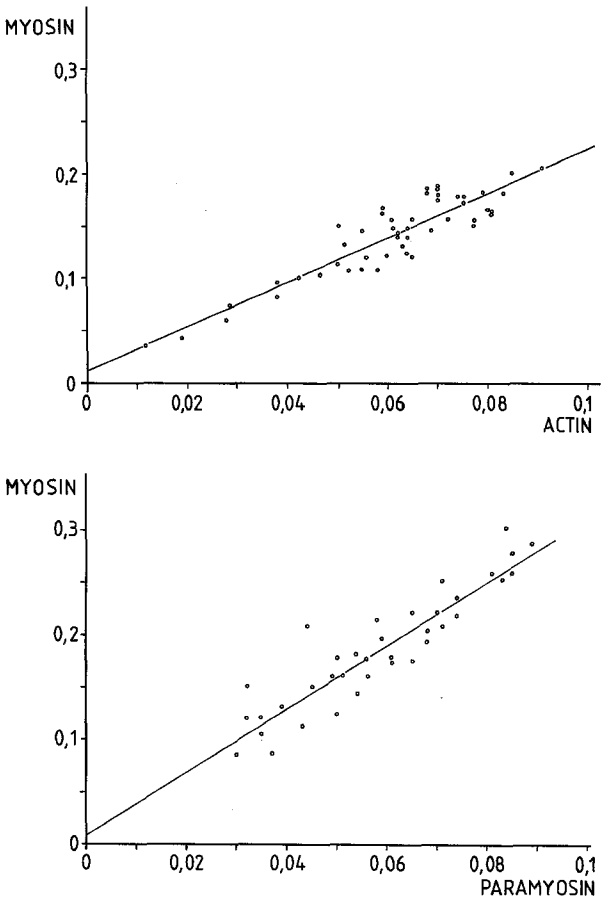

Figure 2. Evaluation of densitograms of 5 % SDS polyacrylamide gels loaded with myofibrillar proteins of honey bee flight muscle. Paper weights (g) of the peak areas of the myosin bands are plotted vs those of the actin bands (upper graph) and the paramyosin bands (lower graph) respectively. Each point represents the measurement of a single gel. The values have been corrected for the different staining behavior of the proteins before plotting. The parameters of the regression lines are $y = 2.31x + 0.011$ (upper graph) and $y = 3.01x + 0.009$ (lower graph).

filament form 12 subfilaments which are grouped in pairs. They are supposed to form the walls of these filaments and to be arranged parallel to their longitudinal axis[8,9]. Filaments with little paramyosin (up to 2 %) seem to be tubular with no additional electron dense structures in the filament core[8]. The cores of filaments with 9–11.5 % paramyosin show additionally three subfilaments[9]. These subfilaments are located concentrically to the myosin subfilaments of the wall at the inner edge of the wall with 3-fold radial symmetry[9]. This arrangement would leave enough space in the filament center for other core proteins such as those which have been proposed by Epstein and coworkers[10] for nematode filaments.

Bee filaments seem to have a different structure. Averaged images of cross-sectioned filaments also showed the three concentrically arranged subfilaments at the edge of the filament wall as mentioned above[9]; however, they seem to contain another set of three subfilaments located eccentrically in the filament core at smaller radii[9].

The coincidence of the double content of paramyosin found in this paper (compared to, e.g., *Lethocerus* filaments[2, 11]) and six subfilaments in the core of bee filaments instead of three (as found in solid myosin filaments of the fleshfly or *Lethocerus*)[9], suggests that the additional paramyosin might be located in the center of the core of the bee filaments, giving rise to the inner set of subfilaments. It would be interesting to know whether there is space left for core proteins other than paramyosin.

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Comparison of lysine and tryptophan catabolizing enzymes in rat and bovine tissues

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Summary. Earlier studies indicate that α-aminoadipate aminotransferase (AadAT) and kynurenine aminotransferase (KAT) activities from rat tissues are associated with a single protein. However, our recent studies indicate that AadAT activity from bovine liver and kidney is not associated with KAT activity. To test whether the lysine and tryptophan catabolism in bovine tissues differ from that in rat tissues, we compared the activities of enzymes involved in lysine and tryptophan pathways in rat and bovine tissues. The activities of lysine catabolizing enzymes such as AadAT, lysine α-ketoglutarate reductase and saccharopine dehydrogenase in the bovine tissues were significantly lower than those found in rat tissues. The activities of tryptophan catabolizing enzymes such as KAT and kynurenine hydroxylase in the bovine tissues were negligible as compared to those in rat tissues. The results suggest that lysine is degraded via the saccharopine pathway in the livers and kidneys of both species but the metabolism of tryptophan in bovine tissues may be different from that in rat tissues.
Key words. Lysine metabolism; tryptophan metabolism; bovine liver; bovine kidney; rat liver, rat liver, rat kidney.

Lysine and tryptophan are the two essential amino acids whose catabolic pathways appear to be interrelated because, they share a common intermediate, α-ketoadipic acid. α-Aminoadipate aminotransferase (AadAT, EC 2.6.1.39) and kynurenine aminotransferase (KAT, EC 2.6.1.7) are the enzymes of lysine and tryptophan catabolic pathways, respectively. Earlier studies in rat tissues indicated that AadAT and KAT are properties of a single protein[2, 3]. However, recently we found that AadAT from bovine kidney was not associated with KAT activity[4]. Another report suggested that in human tissues, the transamination of kynurenine may be similar to that in bovine tissues but different from that in rat tissues[5]. To test whether the lysine and tryptophan catabolism in bovine tissues differ from that in rat tissues, we compared the enzyme activities of the lysine and tryptophan catabolic pathways in kidneys and livers of rat and bovine.

Materials and methods

Chemicals. L-Lysine, L-tryptophan, L-saccharopine, α-ketoglutarate, α-ketoadipate, L-kynurenine sulfate, pyridoxal-5′-phosphate, NADPH and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals used were of analytical grade.

Animals. Five adult male albino Sprague-Dawley rats, weighing 250–300 g were purchased from Charles River Laboratories (Wilmington, ME). Bovine livers and kidneys from five different animals were obtained from a local slaughterhouse where the tissues were removed immediately after sacrificing the animal.

Enzyme assays. Rats were sacrificed by decapitation and their livers and kidneys were immediately removed. Rat and bovine livers or kidneys were homogenized (10 % w/v) in 10 mM Tris-HCl, pH 7.0. The homogenates were centrifuged at 600 × g for 10 min and the resulting super-