

detected in component M_{12} but none in components M_{11} , M_{13} and M_{14} .

It is concluded that the light-chain component of molecular weight 18500 present in skeletal myosin can exist in either a phosphorylated or a non-phosphorylated form. The evidence also suggests that enzymic mechanisms exist for the phosphorylation and dephosphorylation of this light chain.

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A Proteolytic Fragment of 26000 Molecular Weight from the Heavy-Chain Fraction of Myosin

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Subfragment 1 prepared by digestion of rabbit skeletal-muscle myosin by the method of Lowey *et al.* (1969) with high papain/myosin ratios (1:200, by wt.) migrated as two components on polyacrylamide-gel electrophoresis in 40% glycerol in 20mM-tris-glycine buffer, pH8.6. The two components were present in roughly similar amounts. Subfragment 1 prepared by using a papain/myosin ratio 1:2000 contained only a single component with the same electrophoretic mobility as the slower component obtained by digestion with the higher papain/myosin ratio. In each case the band patterns obtained were identical whether the gels were stained for protein or for adenosine triphosphatase activity (Wachstein & Meisel, 1957).

On electrophoresis in 8M-urea in 20mM-tris-glycine buffer, pH8.6, the light-chain pattern of three components (Perrie *et al.*, 1969) was similar in both preparations. The heavy-chain fraction of subfragment 1 was studied by electrophoresis in sodium dodecyl sulphate (Weber & Osborn, 1969). Under these conditions components with molecular weights 15500, 18500 and 23000, which corresponded to the three light-chain components noted on electrophoresis in 8M-urea, could be detected in both preparations. In addition, the digest with the low papain/myosin ratio contained components with molecular weights 87000, 69000 and 26000 derived from the heavy-chain fraction. The digest with the high papain/myosin ratio, however, contained little or none of the '87000 component' but contained a '53000 component' together with the '69000' and '26000 components'. Densitometric traces indicated

that the amount of '26000 component' was higher in preparations that possessed more of the '53000 component'.

The '26000 component' isolated by gel filtration on Sephadex G-200 in sodium dodecyl sulphate migrated as a single band on electrophoresis in sodium dodecyl sulphate. It has an amino acid composition similar to that of the heavy-chain fraction of myosin and contained 0.6mol of mono- N^{ϵ} -methyl-lysine, 0.9mol of tris- N^{ϵ} -methyl-lysine and 0.3mol of 3(*tele*)-methylhistidine/mol of fragment (cf. Hardy *et al.*, 1970).

Thus it appears that, depending on the papain concentration, two species of subfragment 1 molecules are produced that differ mainly in the proteolytic cleavages, present in the heavy chains. It is of interest that, despite these cleavages in the heavy chains, both preparations possess adenosine triphosphatase activity.

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Subunit Structure and Biological Activity of Tropomyosin B from Different Muscle Types

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Tropomyosin is required in addition to the troponin complex to restore Ca^{2+} -sensitivity to the Mg^{2+} -stimulated adenosine triphosphatase of desensitized actomyosin (Ebashi & Kodama, 1966). It also acts independently as an inhibitor of the Ca^{2+} -stimulated adenosine triphosphatase of desensitized actomyosin (Schaub *et al.*, 1967). Whereas the former property is not affected by the blocking of the thiol groups of tropomyosin with iodoacetate, phenylmercuric acetate or bis-2-carboxyethyl disulphide, the latter property was lost after treatment with these reagents. Under defined conditions of pH and ionic strength heating in the range 55–60°C was more effective in destroying the activity of tropomyosin in the relaxing protein system than its inhibitory activity on the Ca^{2+} -stimulated adenosine triphosphatase. Elsewhere we have reported that the former property is more sensitive to controlled mild proteolysis than is the latter (Schaub & Perry, 1969).

In polyacrylamide-gel isoelectric focusing and

electrophoresis in 6M-urea and 15mm-mercapto-ethanol in tris-glycine buffer, pH8.5, tropomyosin from rabbit white skeletal muscle free of the '37000 component' of the troponin complex (Wilkinson *et al.*, 1971) separated into two bands of similar mobility. This pattern was observed on electrophoresis in 6M-urea and in sodium dodecyl sulphate in the presence of thiol compounds and after carboxymethylation. The faster of the two components was estimated to be present in four times the amount of the slower, which, as judged by its radioactivity after carboxymethylation with iodol¹⁴C]acetate, contained half as much cysteine as the more negatively charged component.

Similar two-band patterns were also persistently observed on the electrophoresis of tropomyosin isolated from white skeletal muscle of the cow, sheep and pig. Tropomyosins isolated from rabbit heart and the adductor muscle of *Pecten maximus* migrated as one band only. These findings suggest that tropomyosin preparations may vary according to the type of muscle from which they are isolated. The observation that all tropomyosin preparations possessed both biological activities described above implies that they are associated with one, if not both, of the components observed. The consistent presence of two subunits of different charge and molecular weight (cf. Weber & Osborn, 1960; Sender, 1971; Bodwell, 1967) may explain the primary sequence data of Smillie *et al.* (1971), which imply that rabbit skeletal-muscle tropomyosin may exhibit polymorphism.

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Quaternary Structure of Oligomers with Dihedral Symmetry

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Oligomeric proteins with dihedral symmetry are very common in biological systems. This communication presents formulae for describing all the types of 'perfect' geometrical structures that are theoretically possible for such proteins. The formulae

hold for component protomers of any shape. The approach and terminology of Milner-White (1971) is used. First, the angles θ , ϵ and ζ must be defined.

A point is taken as the 'centre' of the protomer. Any fixed point in a known protomer may be used. Two angles are necessary to describe the geometry of a single site-pair: θ and ϕ . θ is the angle between the centres of three adjacent protomers linked by that site-pair. ϕ need not be defined since for oligomers it is 0°, because the angles of θ in adjacent protomers always lie in the same plane.

Two types of angle are necessary to describe the relationships between two different site-pairs (A and B) on one protomer: ϵ and ζ . ϵ is the angle formed between the lines that bisect the angles θ_A and θ_B . ζ_A is the angle formed by the clockwise rotation (as seen from the outside of the protomer) of the plane of the angle θ_A with respect to the plane of the angle ϵ , measured in a plane perpendicular to the line bisecting ϕ_A . ζ_B is defined similarly. When θ is 0° (the isologous case) its plane is taken as that perpendicular to the dyad axis. Finally the ratio of the distance between the centres joined by site-pair A to the between the centres joined by site-pair B is defined as q .

Three types of site-pairs are theoretically possible in oligomers with dihedral symmetry. Two must be isologous and one heterologous (see Hanson, 1966), except for tetramers in which all are isologous. Any two of these site-pairs are, however, sufficient for a stable structure. Since heterologous binding is apparently rare among proteins (see also Monod *et al.*, 1965) it was suggested (Milner-White, 1971) that the structure with two isologous site-pairs may be the preferred mode of binding in dihedral oligomers. For n -mers of this type (which may in some cases be knotted):

$$\sin^2 \left(\frac{360}{n} \right) \cdot (q^2 - 1) = \sin^2 \epsilon \cdot (q^2 \cdot \sin^2 \zeta_A - \sin^2 \zeta_B)$$

$$\sin^2 \left(\frac{360}{n} \right) \cdot (q^2 + 1 - 2q \cdot \cos \epsilon) = \sin^2 \epsilon \cdot \left[q^2 \cdot \sin^2 \zeta_A + \sin^2 \zeta_B \pm 2q \cdot \sin \zeta_A \cdot \sin \zeta_B \cdot \cos \left(\frac{360}{n} \right) \right]$$

For the case with one isologous and one heterologous (A) site-pair the relationships are:

$$\sin^2 \zeta_A \cdot \sin^2 \epsilon = 1 - \frac{q \cdot \cos \epsilon}{\sin \left(\frac{360}{n} \right)}$$

$$\sin^2 \zeta_B \cdot \sin^2 \epsilon = 1 - \frac{1}{q} \cdot \cos \epsilon \cdot \sin \left(\frac{360}{n} \right)$$

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