The Composition of Microsomal Fractions from Rabbit Skeletal Muscle
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The contraction-relaxation cycle of muscle is regulated by the organized membrane systems of the muscle cell. The cell is enclosed by an outer membrane known as the sarcolemma. Invaginations of the sarcolemma give rise to transverse tubules, which conduct the nerve impulse arriving at the sarcolemma to the neighbourhood of the sarcoplasmic reticulum. The sarcoplasmic reticulum surrounds each myofibril or contractile element and can be divided into three regions, namely, the terminal cisternae, the longitudinal tubules and the fenestrated collar. The terminal cisternae are the sites of calcium storage and release (Winegad, 1968). The sarcoplasmic reticulum links excitation to contraction by regulating the concentration of Ca\(^{2+}\) in the sarcoplasm.

Homogenization of the muscle cell ruptures these membrane systems and results in the formation of rounded-off intact structures known as vesicles. These vesicles retain the properties of their parent membranes. Valuable information on the origin of vesicles can be obtained by using marker enzymes (Headon et al., 1974).

Four concentrated microsomal fractions were prepared and protein and enzyme activities were determined as previously reported (Headon & Duggan, 1971; Headon et al., 1974). Phospholipids were separated by using t.l.c. and determined as described by Hilderson (1974). Cholesterol was measured by using g.l.c. Sodium dodecyl sulphate–polyacrylamide-gel electrophoresis was performed as described by Headon et al. (1974).

In Table 1 the chemical and enzyme compositions of the four microsomal fractions are listed. The protein content of the fractions is high indicating a good yield of microsomes. Ca\(^{2+}\)-uptake activity is greatest in fraction 3 (1.61 \(\mu\)mol of Ca\(^{2+}\)/min per mg of protein at 25°C), followed by fraction 2. Fractions 1 and 4 have lower activities. With succinate dehydrogenase (EC 1.3.99.1) as a marker for the inner membrane of the mitochondrion, contamination by mitochondrial protein was estimated to be approx. 4% in fraction 2 and 5% in fraction 3. Fraction 3 therefore contains highly active Ca\(^{2+}\)-accumulating vesicles, which are relatively uncontaminated by fragmented mitochondria.

The membrane proteins present in these four fractions were solubilized by using sodium dodecyl sulphate. The protein components were separated by using sodium dodecyl sulphate–polyacrylamide-gel electrophoresis and their molecular weights determined. The protein patterns obtained are shown in Fig. 1. All four fractions possess

| Table 1. Protein, lipid and enzyme composition of microsomal fractions |
|--------------------------|----------|----------|----------|----------|
| Fraction no.             | 1        | 2        | 3        | 4        |
| Density of fraction at 5°C| 1.118    | 1.134    | 1.147    | 1.174    |
| Protein concentration (mg/ml) | 1.876    | 1.065    | 1.030    | 0.734    |
| Phospholipid/protein ratio | 0.05     | 0.30     | 0.43     | 0.38     |
| Phospholipid classes (% total phospholipid) |
| Phosphatidylcholine | 26.6     | 64.7     | 65.9     | 50.1     |
| Phosphatidylethanolamine | 50.5     | 13.3     | 13.8     | 17.7     |
| Phosphatidylinositol | 6.9      | 8.7      | 9.6      | 19.5     |
| Minor classes | 15.9     | 13.3     | 10.8     | 12.7     |
| Cholesterol content (\(\mu\)g/mg of protein) | 0.28     | 15.64    | 6.22     | 8.64     |
| Ca\(^{2+}\)-uptake (\(\mu\)mol/min per mg of protein) | 0.147    | 0.986    | 1.609    | 0.640    |
Membrane proteins were solubilized by treatment with sodium dodecyl sulphate. The gel system was calibrated by using proteins of known molecular weight. Full experimental details are given in Headon et al. (1974). → Distance migrated by tracking dye.

Fig. 1. Protein patterns obtained after sodium dodecyl sulphate-polyacrylamide-gel electrophoresis of microsomal fractions

a major protein band with a molecular weight in excess of 100000. When present the Ca$^{2+}$-transport ATPase (adenosine triphosphatase), reported by many workers (Martonosi & Halpin, 1971; MacLennan, 1970; Meissner & Fleischer, 1971; Barrett & Headon, 1974), is found within this protein band. The fractions differ in the minor components present. Fraction 3, having the greatest Ca$^{2+}$-uptake activity, shows strong bands corresponding to molecular weights of 85000, 68000 and 45000. These bands lose intensity in fraction 4 whereas components corresponding to molecular weights of 55000 and 37000 grow in intensity.

Phospholipids are inherent components of biological membranes and therefore are indicators of membrane content. The phospholipid/protein ratio may be used to obtain information on the purity, but not homogeneity of a membrane preparation. A very low ratio results from the presence of a large amount of non-membrane protein. In contrast a very high ratio may indicate the loss of native membrane protein during the preparation. Fractions 2, 3 and 4 have phospholipid/protein ratios typical of membranes. Fraction 3, which has the greatest Ca$^{2+}$-uptake activity, also has the highest phospholipid/protein ratio, the value being 0.43. Fraction 1 has a very low value due to the presence of soluble protein from the zonal supernatant (see method of preparation: Headon et al., 1974). The results of analysis for the percentage composition of the phospholipid classes present in the four fractions are included in Table 1. In fractions 2 and 3 phosphatidylcholine constitutes 64.7% and 65.9% respectively of the total phospholipid whereas phosphatidylethanolamine constitutes approx. 13%. However, in fraction 1 phosphatidylcholine constitutes only 26.6% and phosphatidylethanolamine 50.5% of the total phospholipid. The phosphatidylinositol content increases from 6.9% in fraction 1 to 19.5% in fraction 4 whereas the minor phospholipid classes show little variation between fractions. Clearly phosphatidylcholine is the predominant phospholipid of calcium uptake vesicles.

The significance of cholesterol in biological membranes has now begun to become apparent. It appears that the presence of cholesterol provides a stable membrane framework. Stable membranes such as plasma membranes have a high cholesterol content whereas intracellular membranes have a low cholesterol content (Papahadjopoulos et al., 1973). Evidence on the role of cholesterol in the Ca$^{2+}$-transport process of the
sarcolemmal reticulum has been conflicting (Martonosi, 1968; Drabikowski et al., 1972). Table 1 shows the cholesterol content of each of the four fractions. Fraction 2 with 15.6 μg/mg of protein has far more cholesterol than any of the other fractions. From the data shown it is clear that a high concentration of cholesterol and a high Ca²⁺-uptake activity are not coincident in the same membrane fraction. This result favours the view that cholesterol is not essential for Ca²⁺ transport (Drabikowski et al., 1972).

The four fractions of skeletal muscle microsomes described in the present paper differ in terms of their protein, lipid and enzyme composition. These differences result from membrane heterogeneity.

E. J. B. thanks the Department of Education, Dublin, for a research scholarship.


Proton Movements and Adenosine Triphosphate Synthesis in Rat Liver Mitochondria

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Experiments with O₂-pulsed suspensions of rat liver mitochondria preincubated with a finely ground Dowex cation-exchange resin in the Na⁺ form (D₁Na) have given two main results: (i), the pH decrease (−ΔpH), measured by a glass electrode which customarily follows an O₂-pulse, can be entirely abolished and (ii), ATP synthesis continues, though it may be diminished by amounts from 40–70% (Fig. 1). These findings raise the question of the significance of glass-electrode measurements in pulse experiments and, even more basically, of the relation between the postulated proton movements of the chemiosmotic hypothesis and synthesis (Mitchell & Moyle, 1967). The hypothesis claims that both electroneutral (Eₐ) and electrogenic (E₈) proton movements are monitored by a glass electrode (Mitchell & Moyle, 1969); this ready dispersion in vitro of the synthetic force (Δp) could indicate an inefficient process in vivo, or unphysiological conditions in vitro, or a fault in the chemiosmotic argument.

D₁Na, like the glass electrode, is wholly extramitochondrial and from the abolition of the pH spike without the abolition of synthesis we draw two main conclusions. (a) Only Eₐ(Ca²⁺/H⁺) exchange is directly affected by D₁Na and (b) ATP synthesis is affected indirectly. Our experiments require a clear distinction between the distribution of protons moved across the coupling membrane by Eₐ and E₈ exchange; they also require a relationship between these movements. In formulating this distinction we follow closely