Glycogen as a Fuel for Skeletal Muscle

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The utilization of glycogen by skeletal muscle was, at one time, one of the central problems of biochemistry. Attention focused on the most dramatic property of the system: the very rapid glycogenolysis that occurs synchronously with muscle contraction. This phenomenon now has a generally accepted explanation: the increase in Ca\(^{2+}\) that induces muscle contraction also activates phosphorylase kinase. This enzyme catalyses the phosphorylation of inactive phosphorylase \(b\) to active phosphorylase \(a\) and this, finally, catalyses glycogenolysis. Another mechanism whereby an extrinsic stimulus catalyses glycogenolysis is also well understood. Circulating hormones indirectly activate cyclic AMP-dependent protein kinase, which catalyses phosphorylation of phosphorylase kinase to a more active form, which functions at lower Ca\(^{2+}\) concentration. Graves & Wang (1972) have reviewed these pathways.

Another mechanism has been extensively studied \textit{in vitro} with purified phosphorylase, but is less well understood \textit{in vivo}. This involves non-covalent activation of phosphorylase \(b\) by binding of AMP or IMP. Both these activators accumulate in energy-depleted tissues, whereas two inhibitory nucleotides, ATP and ADP, predominate in energy-rich tissues. Glucose 6-phosphate, the enzyme’s effective product, is also a potent inhibitor, and somewhat less effective inhibition is provided by UDP-glucose, the substrate for glycogen synthesis. This system, shown in Scheme 1, would provide a mechanism whereby a constant concentration of glucose 6-phosphate may be maintained in skeletal muscle by glycogenolysis to supply the metabolic needs of the muscle cell.

\textbf{Phosphorylase kinase deficiency}

The properties of purified phosphorylase have been extensively studied and a complex allosteric and co-operative mechanism has been discovered, involving the activators and inhibitors already mentioned and the immediate substrates and products, glycogen, \(P_i\), and glucose 1-phosphate (see Graves & Wang, 1972). Most of this work has been performed on purified rabbit enzyme and, unless otherwise stated, parameters quoted refer to this enzyme source. The physiological significance of this non-covalent activation system is not yet clear. That it has a physiological significance is most strongly suggested by the phosphorylase kinase-deficiency mutation in mice, which is compatible with a normal life (Lyon & Porter, 1963). These mice degrade glycogen on exercise despite a total absence of phosphorylase \(a\) in their skeletal muscles (Danforth & Lyon, 1964), and it has been generally assumed that they do so by non-covalent activation of phosphorylase \(b\). Danforth & Lyon (1964) showed a lag in the rate of accumulation of glucose 6-phosphate and lactate (which they used as an index of glycogenolysis) when isolated muscles of these mice were tetanically stimulated. This lag would be expected if glycogenolysis were dependent on accumulation of AMP or IMP.

\textit{AMP-induced activation of phosphorylase \(b\)}

It has normally been assumed that non-covalent activation of phosphorylase \(b\) would depend on AMP binding. This was partly because AMP activates at much lower
concentrations than IMP ($K_a$ 20–100$\mu$M instead of 2mM) and induces positive homotropic and heterotropic co-operativity, but also because early estimates of muscle AMP concentration were unduly high and it was thought that muscle work would raise it still higher. Fischer et al. (1971), for instance, in their discussion of non-covalently induced phosphorylase $b$ activity in resting skeletal muscle, assumed an AMP concentration of 500$\mu$M. More recent estimations of total muscle AMP have been below 100$\mu$M; we found 94$\mu$M in resting and 57$\mu$M in tetanically stimulated normal mouse muscle. In an effort to induce the expected rise in AMP concentration, we maintained the tetanus for 120s, but there was no significant change.

There is also evidence that AMP is compartmented in skeletal muscle and that the free AMP concentration, at least in one compartment, must be a small proportion of the total. This arises from the observation that most of the approx. 1mM-ADP found in acid extracts of skeletal muscle remains bound to protein if gentler extraction procedures are used (Seraydarian et al., 1962). Much of this bound ADP (approx. 0.6mM) is known to form part of F-actin, and some of the remainder would be bound to myosin. Mommaerts et al. (1975) consider that only 30$\mu$M-ADP would be free in solution in resting frog muscle.
The total ATP concentration, on the other hand, is thought to approximate to its free concentration. This is because ATP is present at much higher concentration than any plausible binding-site pool and because no significant pool of high-affinity ATP-binding sites is known. If, then, the free ATP and ADP concentrations are known, and if they are assumed to be in a single compartment, maintained at chemical equilibrium by adenylate kinase catalysis, then the free concentration of AMP can be calculated from the equilibrium constant. Beis (1973), Rahim et al. (1976) and Mommaerts et al. (1975) have made such calculations, and Rahim et al. (1976) have found 3.4 μM-AMP in muscle of normally active mice and 4.8 μM-AMP after exercise.

A more sophisticated calculation, which allows for binding of H+ and Mg2+ to the three adenylates (but which requires an assumption as to pH and free Mg2+ in muscle), was made by Goodman & Lowenstein (1977) using equations developed by McGilvery & Murray (1974). They found 0.2 μM-free AMP and 33 μM-free ADP in resting and 1.0 μM-free AMP and 62 μM-free ADP in working muscle. If these very low free AMP concentrations were present in the same compartment as phosphorylase b, they would only slightly activate the enzyme, even in the absence of inhibitors.

**IMP-induced activation of phosphorylase b**

This surprising constancy in muscle AMP concentration is explained by the marked increase in IMP which occurs in working muscle. We have found a 100-fold rise, from 18 μM in resting normal muscle to 1.8 mM after 60 s tetanus. Phosphorylase kinase-deficient muscle has a higher resting IMP concentration (48 μM) and it shows a 6-fold more rapid rise during tetanus, reaching 2.3 mM after 10 s. These IMP concentrations in tetanized muscle would activate phosphorylase b to approx. 50% of \( V_{\text{max}} \), if no inhibitors were present.

Since phosphorylase is present at very high activity in skeletal muscle, it need only be slightly activated to achieve an intense glycogenolysis. IMP-induced activation could thus account for the glycogenolysis in exercising phosphorylase kinase-deficient mice and would also tend to promote glycogenolysis on exercise in normal animals.

**Inhibitors of non-covalent activation**

The degree to which phosphorylase b can be activated non-covalently depends, also, on the concentrations of inhibitors present. The free ADP concentration has already been discussed; it rises 2-fold to approximately half its inhibitory constant on exercise (Goodman & Lowenstein, 1977). ATP concentration fell from 12 to 9 mM after 120 s tetanus in our experiments. This fall would have little effect on phosphorylase b activity, since the inhibitory constant for ATP is approx. 3 mM. UDP-glucose can probably be neglected, since its concentration, even in resting muscle, is much below its inhibitory constant (Piras & Staneloni, 1969; Madsen, 1961).

The remaining inhibitor, glucose 6-phosphate, has a complex action. Whereas ATP and ADP compete for the same binding site as AMP and IMP (Griffiths et al., 1976), glucose 6-phosphate appears to bind elsewhere. To a first approximation it can be regarded as competing formally with the nucleotide activators (Griffiths et al., 1976). The glucose 6-phosphate concentration in normal mouse muscle rose from 0.3 to 6 mM after 60 s tetanus. The enhancement of inhibition of phosphorylase b may be still greater, since Battersby & Radda (1976) showed that only the \( \alpha \)-anomer of glucose 6-phosphate acts as an inhibitor. This anomer forms 36.5% of a solution at equilibrium, but glucose 6-phosphate freshly produced from glycogen will probably contain a higher proportion, since the \( \alpha \)-conformation may be retained after glycogenolysis. We found that glucose 6-phosphate in phosphorylase kinase-deficient muscle was maintained at a lower
concentration during tetanus. It was 2 mm after 90s tetanus, although the fall in glycogen concentration was normal or supranormal in these animals.

Glucose 1-phosphate and glucose 6-phosphate are kept near equilibrium in vivo by phosphoglucomutase. The equilibrium is heavily in favour of glucose 6-phosphate, which is thus, for many purposes, the effective product of glycogenolysis. The pathways that utilize glucose 6-phosphate, particularly glycolysis in the present context, are controlled separately so that the pool of glucose 6-phosphate acts as a source of substrate for all of them. The smaller pool size in phosphorylase kinase-deficient muscle would minimize inhibition of phosphorylase b (and, indeed, of hexokinase) and might form part of the adaptation of these mice to reliance on non-covalent phosphorylase b activation.

Substrate concentrations and phosphorylase b activity

The concentrations of the substrates of phosphorylase b clearly play a major role in determining the rate of its reaction. Glycogen is present in normal mouse muscle in saturating concentrations, and, even after 120s tetanus, 27% remains. We have found, however, that 34% of the glycogen extracted from mouse muscle is not degraded by phosphorylase so that the rate at which glycogen can be debranched probably becomes important in prolonged tetani. In phosphorylase kinase-deficient muscle the glycogen concentration is much higher (10.7 and 2.3 mg/g respectively) and even after 120s tetanus only 38% is degraded. Since 50% of glycogen extracted from these kinase-deficient muscles can be degraded by purified phosphorylase, it appears that the rate of debranching does not become critical even during these prolonged tetani.

It is not easy to measure Pi in skeletal muscle because of the presence of high concentrations of organic phosphates, which are hydrolysed by vigorous extraction procedures. More gentle procedures may fail to inactivate the many powerful phosphatases in the tissue. Dawson et al. (1977) used their non-destructive 31P n.m.r. technique to determine Pi and found approx. 1 mm. The same group have found evidence for phosphate compartmentation in intact muscle (Seeley et al., 1976). The rise in phosphate concentration in exercising intact muscle has not been quantified by n.m.r., but must be considerable, since we found a 16 mm fall in phosphocreatine concentration after 10s tetanus. Although some of this phosphate will be taken up by formation of glucose 6-phosphate and other phosphorylated intermediates, it is likely that a rise in Pi plays an important role in muscle glycogenolysis during exercise.

Physiological role of non-covalent phosphorylase activation

The role of AMP- or IMP-stimulated phosphorylase b activation in normal muscle is probably concerned primarily with glycogenolysis in response to the muscle's internal needs. Since the IMP concentration rises during muscle contraction, phosphorylase b activity would tend to be enhanced, but this tendency would be opposed by the rise in glucose 6-phosphate and free ADP concentrations. Phosphorylase a, which is formed in response to neural stimulation of muscle, is not inhibited by ADP or glucose 6-phosphate, so its action would predominate. In prolonged tetanic stimulation of muscle the phosphorylase a content falls to very low values (Piras & Staneloni, 1969) and non-covalently activated phosphorylase b would then predominate. It should be remembered, however, that most of the glycogen branches accessible to phosphorylase would have been degraded at this stage and that the activity of debranching enzyme could become rate-limiting.

If the primary role of non-covalently activated phosphorylase b is, indeed, to degrade glycogen in response to the cell's internal needs it may be difficult to study this system experimentally. The glycogenolysis would tend to be much smaller than that induced by muscle contraction and variations in glucose uptake could cause confusion. It would also be difficult to exclude the possibility that small amounts of
phosphorylase \( a \) had been degraded during extraction, even if phosphorylase inhibitors were used.

The simplest system for studying non-covalent phosphorylase \( b \) activation remains phosphorylase kinase-deficient mouse muscle. The glycogen content of this muscle falls rapidly in the first 30s of tetanus, but glycogenolysis then slows and maintains a steady rate of 0.019 mg of glucose/s per g for a further 90s tetanus. This corresponds to 6\% of \( V_{\text{max}} \) for phosphorylase in this tissue. The concentrations of substrates, activators and inhibitors all remain fairly stable in this period and it should, in principle, be possible to calculate the theoretical activity of the enzyme. We have made such a calculation, using the approximation that glucose 6-phosphate competitively inhibits AMP and IMP binding, and found a theoretical activity of approx. 10\%. Further studies of this kind will, however, require a more detailed knowledge of the behaviour of mouse muscle phosphorylase \( b \).

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**Glucose as a Fuel for Small Intestine**

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This account is limited to an outline of the possible role of glucose as a fuel for the non-ruminant small intestine. Glucose entering the cells of other tissues discussed in this colloquium does so solely from the vascular supply; intestine has access to plasma glucose, but also receives postprandial supplies of carbohydrate into its lumen. Understanding of the issues to be discussed thus requires preliminary consideration of five points peculiar to intestinal metabolism: (1) the structure of small intestine, in particular its minimum physiologically competent component, the villus (Fig. 1); (2) the quantity and variety of carbohydrate entering the lumen of the small intestine (a typical European or North American daily diet includes 150g of starch and glycogen, 120g of sucrose, 30g of lactose and 10g of monosaccharide); (3) the daily digestion of 150g of disaccharides, and the terminal stages of digestion of 150g of polysaccharide, by amylglucosidase, maltase, isomaltase, sucrase and lactase located in the luminal pole (brush border) of the columnar absorptive cells (Fig. 1) constitutes one mode of entry of dietary carbohydrate from the intestinal lumen into these cells (Storelli et al., 1972) and