THE ROLE OF PHOSPHOLIPIDS IN THE ATP-ASE ACTIVITY OF SKELETAL MUSCLE MICROSOMES

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Skeletal muscle microsomes actively transport Ca^{++} if Mg^{++} and ATP are present (Ebashi and Lipmann, 1962; Hasselbach and Makinose, 1961). Hasselbach (1964) proposed that the transport of Ca^{++} is mediated by a carrier system which, in its phosphorylated form, binds Ca^{++} with high affinity. Indications for the existence of the intermediate were provided recently by Yamamoto and Tonomura (1967).

The ATP-ase activity and Ca^{++} transport are inhibited by treatment of microsomes with phospholipase C, parallel with the hydrolysis of lecithin. Both functions are reactivated by micellar dispersions of synthetic phospholipids (Martonosi, 1964). It is likely that lecithin is required for the phosphorylation or dephosphorylation of the proposed carrier.

In order to settle this question the steady state concentration of phosphorylated intermediate was measured under various experimental conditions on control and phospholipase C treated

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microsomes, using ATP^{32} . The data suggest a lecithin requirement in the dephosphorylation step.

Methods and Materials. The isolation of microsomes and their treatment with phospholipase C, the determination of proteins, and radioactivity were carried out as described earlier (Martonosi, 1963; 1964). ATP^{32}, labelled predominantly in the terminal position, was prepared as described by Lowenstein (1957), and purified by chromatography on Dowex-1-HCO_3 column (Martonosi, 1960). $KHCO_3$ was removed with Dowex-50 resin in H^+ form. Incubation of microsomes with ATP^{32} was carried out at 25 C for 20 seconds in the following solution: 0.05 M KCl, 0.005 M histidine, 1.75×10^{-6} M ATP^{32}, with other additions as indicated in the Legends. Concentration of microsomal proteins was 0.3-1.0 mg./ml. The reaction was stopped, and the labelled membranes were isolated according to Post, Sen and Rosenthal (1965). ATP-ase activity was estimated from the amount of P_i^{32} present in the charcoal treated supernatant (Post et al., 1965). Less than 30% of the ATP was hydrolysed in any of the reported experiments.

Results. An acid-stable, membrane-bound phosphate intermediate (PI) is formed on incubation of microsomes with ATP^{32} which is not extracted by lipid solvents from the membrane protein.

The steady-state concentration of PI is influenced by the ionic composition of the medium as shown in Table 1.

With 5 mM $MgCl_2$ the PI concentration is about 0.003 mole/10^7 g. protein; it reaches 6-8 moles/10^7 g. protein in the presence of 5 mM $CaCl_2$. In mixtures of 5 mM $MgCl_2$ and increasing concentrations of Ca^{++} the ATP-ase activity and the steady state concentration of PI vary inversely. Maximum ATP-ase and minimum PI concentrations were observed at 10^{-5}-10^{-4} M $CaCl_2$. Na^+ and

	Additions	Moles of P^{32} bound/10^7 g. protein	
		Control microsomes	Phospholipase C treated microsomes
1	$MgCl_2$	0.003	2.220
2	$MgCl_2$ + EGTA	0.036	1.640
3	$MgCl_2$ + EGTA + ADP	0.003	0.043
4	$MgCl_2$ + EGTA + 0.05% Triton-X-100	0.029	0.037
5	$CaCl_2$	1.280	6.000
6	$CaCl_2$ + ADP	0.041	0.115
7	$CaCl_2$ + 0.05% Triton-X-100	5.840	4.450

Table 1. Effect of phospholipase C treatment on the concentration of membrane-bound phosphate intermediate. The final concentrations of Mg^{++}, Ca^{++}, ADP and EGTA (ethyleneglycol-bis (β amino-ethyl-ether)-N,N'-tetraacetate) were 5 mM. Protein concentration was 0.9 mg./ml. For other details see Methods and Materials.

K^+ were without significant effect under conditions similar to those used by Post et al. (1965).

With 5 mM $MgCl_2$ as activator, the concentration of PI is 10^2-10^3 times higher in phospholipase C treated than in control microsomes. The high PI concentrations of lecithin-depleted microsomes are reduced to control levels in the presence of Mg^{++} by treatment with synthetic dipalmitoyl-lecithin (Fig. 1) or Triton-X-100 (Table 1).

With 5 mM $CaCl_2$ as activator, the difference between control and phospholipase C treated microsomes is less pronounced and neither Triton-X-100, nor addition of phospholipids lowers markedly the protein-bound radioactivity (Table 1).

ADP and less effectively IDP can serve as phosphate accep-

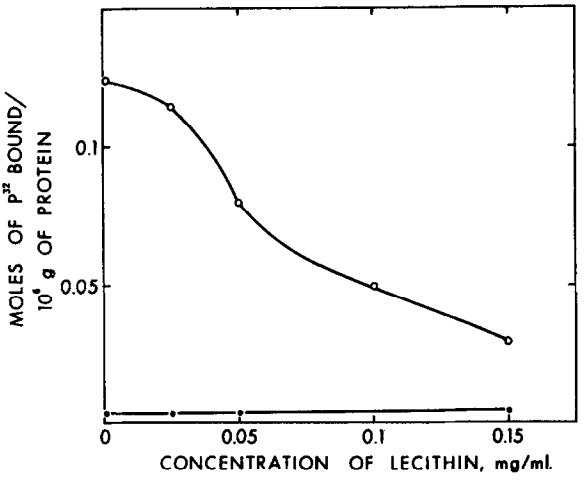

Fig. 1. Effect of lecithin on the concentration of phosphorylated intermediate. Assay system contained 5 mM $MgCl_2$, 5 mM EGTA and ultrasonically dispersed suspension of synthetic dipalmitoyllecithin (Sigma) as indicated, in addition to the standard assay constituents. Microsomes were incubated in the media for one minute prior to starting the reaction with ATP^{32}.
●-● control microsomes; o-o phospholipase C treated microsomes.

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tors reducing the protein-bound P^{32} to low levels in both control and lecithin-depleted microsomes, using either Mg^{++} or Ca^{++} as activators.

The massive accumulation of PI in phospholipase C treated microsomes with Mg^{++} as activator suggests the involvement of lecithin in the dephosphorylation step of ATP hydrolysis. If in the lecithin-depleted membrane the postulated movement of the phosphorylated carrier from the outside to the inside membrane surface cannot occur, its dephosphorylation will not take place, and the phosphorylated form will accumulate with concomitant inhibition of ATP-ase activity and Ca^{++} transport.

Phospholipids (and some detergents) appear to restore membrane structure sufficiently to permit carrier movement, with

the accompaning phosphorylation - dephosphorylation cycle, and Ca^{++} transport.

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