BBA 43217

Activation of Ca^{2+} uptake by acetyl phosphate in muscle microsomes

BADER AND SEN[1] and YOSHIDA, IZUMI AND NAGAI[2] have shown a K^+-dependent acetyl phosphatase activity in microsome preparations of guinea pig kidney cortex and brain. On the basis of these observations, a possible role of acetyl phosphate in the Ca^{2+}-transport system of skeletal muscle microsomes was investigated.

Skeletal muscle microsomes were prepared as previously described[3] and stored at —5° until use. All preparations used were less than 36 h old. In a standard assay, the incubation medium consisted of 8 mM Tris–maleic acid buffer (pH 6.8), 4 mM $MgSO_4$, 0.1 mM $^{45}CaCl_2$, 2 mM Tris–oxalate, 120 mM KCl, and specified amounts of acetyl phosphate. The total volume was usually 2.5 ml. The reaction was started by the addition of microsomes (total of 1 mg protein) and stopped after 5-min incubation at 37° by filtering the mixture through a Millipore filter (type HA; average pore size, 0.45 μ). In all experiments, control experiments without microsomes or with microsomes but without acetyl phosphate were performed. 0.05-ml aliquots of the filtrates were dried on a planchette and counted in a gas-flow counter. The percentage of Ca^{2+} bound to microsomes was calculated from the radioactivity of the incubation medium without microsomes (A) and that with microsomes (B), according to the relationship: per cent of Ca^{2+} bound = 100 × (A—B)/A. The acetyl phosphatase activity was determined by measuring the unhydrolyzed acetyl phosphate in the filtrate as the hydroxamate by the method of Lipmann and Tuttle. The dilithium salt of acetyl phosphate (Sigma Chemical Co.) was converted to the Tris salt by passing it over a cooled Dowex 50W-X8 column in Tris form and then neutralizing with 1 M Tris. $CaCl_2$ was purchased from Abbott Radio-Pharmaceuticals. All the other reagents were of analytical grade.

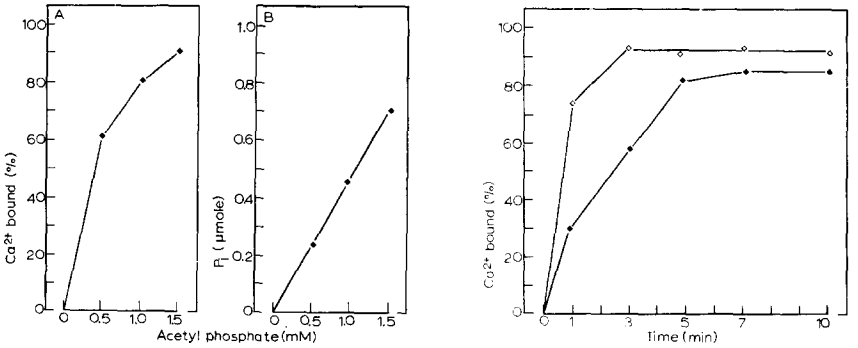

Fig. 1. Activation of Ca^{2+} uptake by acetyl phosphate. The incubation medium was as described in the text; incubation time, 5 min. The reaction was started by the addition of microsomes (total of 1 mg protein). A. Ca^{2+} uptake. The figure shows a typical experiment. Similar results were observed in 20 different microsome preparations tested. B. Acetyl phosphatase activity in the same experiment of A.

Fig. 2. Ca^{2+} uptake at different incubation intervals. The incubation medium was as described in the text; final volume of the mixture, 10 ml. The reaction was started by the addition of microsomes (total of 4 mg protein). Aliquots were filtered after different periods of incubation at 37°. ◇, ATP, final concentration in the reaction medium, 0.20 mM; ◆, acetyl phosphate, final concentration in the reaction medium 0.30 mM. Similar results were observed in 4 different microsome preparations tested.

Fig. 1 shows that skeletal muscle microsomes hydrolyze acetyl phosphate. Figs. 1 and 2 show that acetyl phosphate can also be used as substrate for microsomal Ca^{2+} uptake. Fig. 2 shows that acetyl phosphate is a less efficient substrate than ATP, since larger amounts are required and the rate of Ca^{2+} uptake is slower in the acetyl phosphate-containing system.

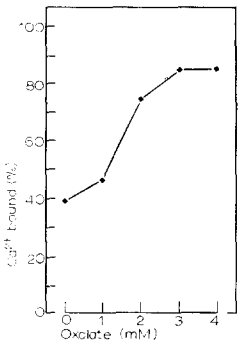

Fig. 3. Oxalate dependence. The incubation medium was as described in the text except for the oxalate concentration. Acetyl phosphate concentration, 0.30 mM. The reaction was started by the addition of microsomes (1 mg protein). Incubation was for 5 min at 37°. Similar results were observed in 4 different microsome preparations tested.

Different reports have shown that oxalate–Tris potentiates microsomal Ca^{2+} uptake through the precipitation of Ca^{2+} inside the microsomal structure[3,4]. In other words, oxalate increases the microsomal Ca^{2+} retention capacity. Fig. 3 shows that oxalate–Tris also potentiates microsomal Ca^{2+} uptake when acetyl phosphate is used as substrate.

$HgCl_2$, at a concentration of $4 \cdot 10^{-5}$ M, completely inhibits microsomal Ca^{2+} uptake when either ATP or acetyl phosphate is used as substrate. Ouabain, up to a concentration of 1 mM, did not inhibit microsomal Ca^{2+} uptake.

In conclusion, the data presented show that acetyl phosphate can substitute ATP as a substrate for microsomal Ca^{2+} uptake.

This investigation was supported by the "Conselho Nacional de Pesquisas", "Conselho de Pesquisas da Universidade Federal do Rio de Janeiro" and by Grant 5 R05 TW00181-03 from the National Institutes of Health. The author is grateful to Mr. ISALTINO R. SOARES for his excellent technical assistance.

Instituto de Biofisica,
Universidade Federal do Rio de Janeiro,
Rio de Janeiro (Brazil)

LEOPOLDO DE MEIS

1 H. BADER AND A. K. SEN, *Biochim. Biophys. Acta*, 118 (1966) 116.
2 H. YOSHIDA, F. IZUMI AND K. NAGAI, *Biochim. Biophys. Acta*, 120 (1966) 183.
3 L. DE MEIS, *J. Biol. Chem.*, 243 (1968) 1174.
4 A. MARTONOSI AND R. FERETOS, *J. Biol. Chem.*, 239 (1964) 648.

Received September 16th, 1968