ATP AND ACTIVE TRANSPORT

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For about a year it is known that a suspension of the isolated "endoplasmatic reticulum" of skeletal muscle (relaxing granules) in an appropriate calcium free oxalate solution splits ATP at a low rate. The splitting is insensitive to salyrgan*; it is called "basic" splitting (Hasselbach und Makinose 1961).

For just as long it is known that calcium added to such systems is pumped into the granules and stored there in extraordinarily great amounts and with very high rate. From the moment when calcium is added, to the moment when all calcium is stored, the ATP is simultaneously split at a rate 7 to 8 times higher than in calcium free solutions. This increase in splitting is called "extra" splitting (Hasselbach and Makinose 1961).

The storage of calcium and the "extra" splitting are completely inhibited by the same concentration of salyrgan. Since the "extra" splitting is sensitive to salyrgan while the "basic" splitting is insensitive to the latter it is reasonable to conclude that in both cases two different ATPases are concerned. The parallelism between the storage of calcium and the activity of the "extra" ATPase makes it most likely

*Salyrgan: Salicyl-hydroxymercuri-methoxypropyl-amidoorthoacetate.

that the "extra" splitting is the source of energy necessary for storage.

The fact that the "extra" splitting ceases as soon as the calcium concentration "outside" decreases to 10^{-6}M suggests that the calcium activated step of the "extra" splitting takes place on the outer side of the membrane. "Inside" the concentration of the dissolved calcium is constant $2 \cdot 10^{-4}$M (in equilibrium with the calcium oxalate crystals).

The "basic" ATP splitting, apart from an initial period of 1 to 2 minutes, is not connected with a phosphate exchange between ATP and ADP (Fig. 1).

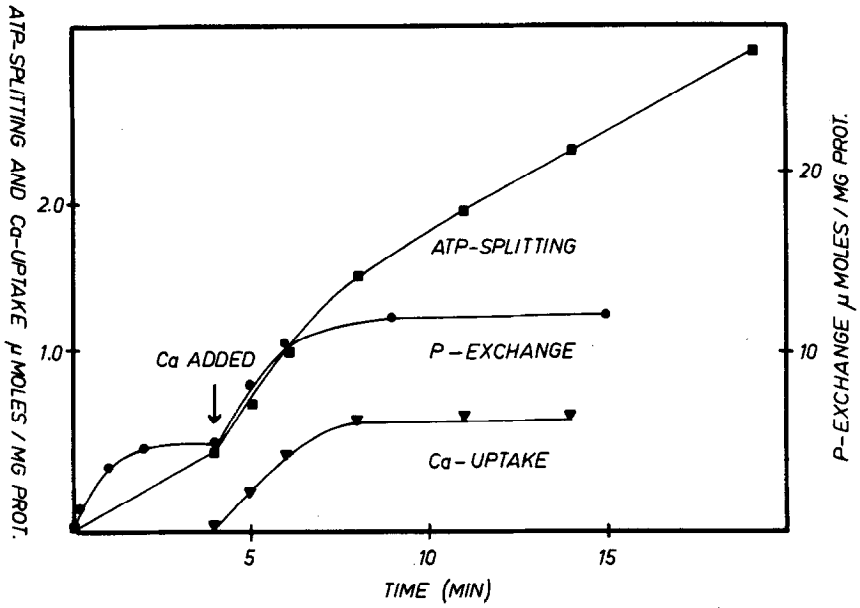

Fig. 1. ATP "extra" splitting, calcium uptake and phosphate exchange.
Mg^{++}= oxalate = ATP =5.10^{-3}M, μ =0,1, histidine= 0,01M pH =7,0, T =20°C, 0,46 mg prot/ml.

However as soon as the uptake of calcium is started by addition of calcium to the solutions containing oxalate all at once a phosphate exchange between ATP

and ADP sets in which ceases equally abruptly when all the calcium is stored in the granules (Fig. 1). Consequently, in proportion to the amount of the added calcium not only the amount of the phosphate liberated increases but also that of the exchanged phosphate. The rate of the calcium induced phosphate exchange during the storage of calcium is ten times as great as the rate of the "extra" splitting occuring simultaneously (Fig. 1).

If the calcium pump and the "extra" ATPase are inactivated by the addition of salyrgan (5 μ moles/mg prot.) or oleic acid (0,2 μ moles/mg prot.) even in the absence of calcium a certain exchange of phosphate takes place (Tab. 1).

Table 1

Suppression of the calcium activation by salyrgan and oleic acid

Inhibitor	μ moles per mg prot. and minute				
	P exchange		ATP splitting		Ca uptake
	absent	present	1,2 $.10^{-4}$M Ca absent	present	present
nil	0	2,3	0,09	0,28	0,20
Salyrgan*	0,8	0,8	0,08	0,08	0
Oleic acid*	1,2	1,4	0,3	0,3	0

* without ADP, conditions as in Fig. 1

Yet, this phosphate exchange, which cannot be explained at the present, cannot be increased by the addition of calcium (Tab.1). By ADP the calcium induced exchange is inhibited to about the same extent as the uptake of calcium as well as the "extra"-splitting (Fig.2).

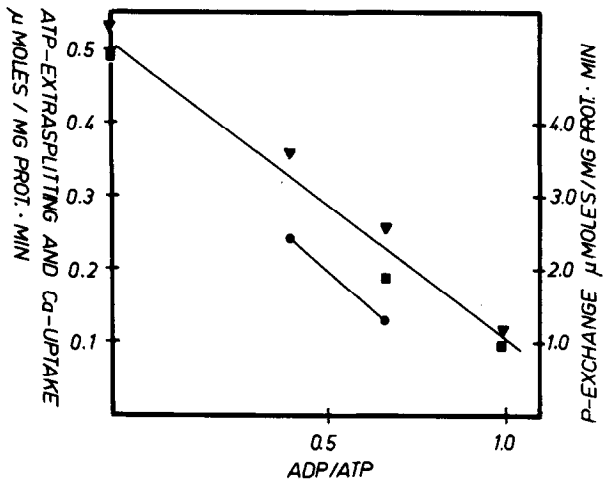

Fig.2. The inhibition produced by ADP. Calcium uptake ▼, "extra"-splitting ●, phosphate exchange ■, 0,2 - 0,3 mg prot./ml, other conditions as in Fig.1.

Hence, the activity of calciumpump, "extra"-ATPase and phosphate exchange are strictly correlated. This fact suggests that the calcium induced "extra"-splitting is initiated by the transfer of the phosphate of the ATP into an "energy-rich" bond of an unknown substance. This process must take place on the outer surface of the membrane of the granule, because it depends on the presence of calcium in the outer solution. The findings reported are in accordance with the usual conceptions concerning the mechanism of the active transport (cf. Järnefelt, 1961) if we assume the following:

1. The unknown substance mentioned becomes a carrier by phosphorylation on the outer surface of the granules which increases greatly its affinity for calcium.
2. The calcium complex of the phosphorylated carrier diffuses to the inner surface of the membrane of the granule. There the phosphate group is split off from

the carrier whereby the calcium affinity of the carrier is strongly diminished. Thus the bound calcium is liberated in spite of the comparatively high concentration of calcium "inside". This type of transport is energetically possible, because not more than 20 to 30 percent of the energy content of the terminal phosphate bond of ATP is converted into concentration work.

References

Hasselbach,W. und M.Makinose, Biochem.Z. 333, 518(1961).

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