THE INITIAL PHASE OF Ca^{2+}-UPTAKE AND ATPase ACTIVITY OF SARCOPLASMIC RETICULUM VESICLES

Mark KURZMACK and Giuseppe INESI
Laboratory of Physiology and Biophysics, University of the Pacific, San Francisco, California 94115, USA

Received 10 December 1976

1. Introduction

An initial burst in the course of P_i production by sarcoplasmic reticulum vesicles (SR) was first pointed out by Kanazawa et al. [1] and attributed to a transition in the rate constant for hydrolysis of a phosphorylated enzyme intermediate ($E\sim P$). Subsequently, an initial burst was observed by Froehlich and Taylor [2] and related to formation of a second, acid-labile phosphorylated intermediate ($E\cdot P$). The reported measurements were made in the presence of low ATP concentrations, or limited to the first 300 ms of reaction. We have now obtained resolution of the entire curve of P_i production within a time interval between 20 ms and 10 s in the presence of ATP concentrations producing near maximal velocity at 25°C. In addition, we have monitored the simultaneous occurrence of Ca^{2+}-uptake determining, thereby, Ca/P_i coupling ratios in the transient state.

2. Methods

SR was prepared from rabbit skeletal muscle as previously described [3]. Reaction mixtures for ATPase activity were mixed and quenched with a Durrum D-133 multimixing apparatus. Acid-stable phosphoprotein and P_i were determined as described by Froehlich and Taylor [2]. Ca^{2+}-uptake by SR was measured by following the light absorption changes undergone by the metallochromic indicator murexide, after mixing the reagents by stopped flow [4].

3. Results and discussion

Addition of ATP (0.5 mM) to a mixture containing SR, Ca^{2+} and required cofactors results in P_i production displaying an initial burst within the first 100 ms of reaction, and then proceeding at decreasing velocity (fig.1, top). The initial burst is also obtained in the absence of Ca^{2+} (1.0 mM EGTA and no added calcium). In this case however, the activity following the burst proceeds linearly and is lower than in the presence of Ca^{2+}.

It is apparent that the initial P_i burst is of similar magnitude in the absence and in the presence of Ca^{2+}. However, precise estimates of the initial burst in the presence of Ca^{2+} are hampered by the complexity of the time curve obtained in this condition. On the other hand, the magnitude of the burst in the absence of Ca^{2+} may be reliably estimated by extrapolation of the linear activity observed in this condition (fig.1, top).

The initial P_i burst observed by us evidently corresponds to that described by Froehlich and Taylor [2], but is of greater magnitude (18 versus 3 nmol/mg protein) due to the higher ATP concentrations used in our experiments. The dependence of the initial P_i burst on the ATP concentration is reported in table 1.

The catalytic mechanism of SR ATPase includes an acid-stable phosphorylated enzyme intermediate ($E\sim P$), which can be demonstrated by incorporation of the ATP terminal [^{32}P]phosphate [5]. It was suggested by Froehlich and Taylor [5] that the initial P_i burst corresponds to the formation of an acid-labile phosphate enzyme complex ($E\cdot P$) which is

cleaved upon quenching the reaction at acid pH. This complex would represent a further intermediate step in the mechanism of ATP hydrolysis as follows:

$$E+S \longleftrightarrow ES \longleftrightarrow E\sim P \longleftrightarrow E\cdot P \rightarrow E+P$$

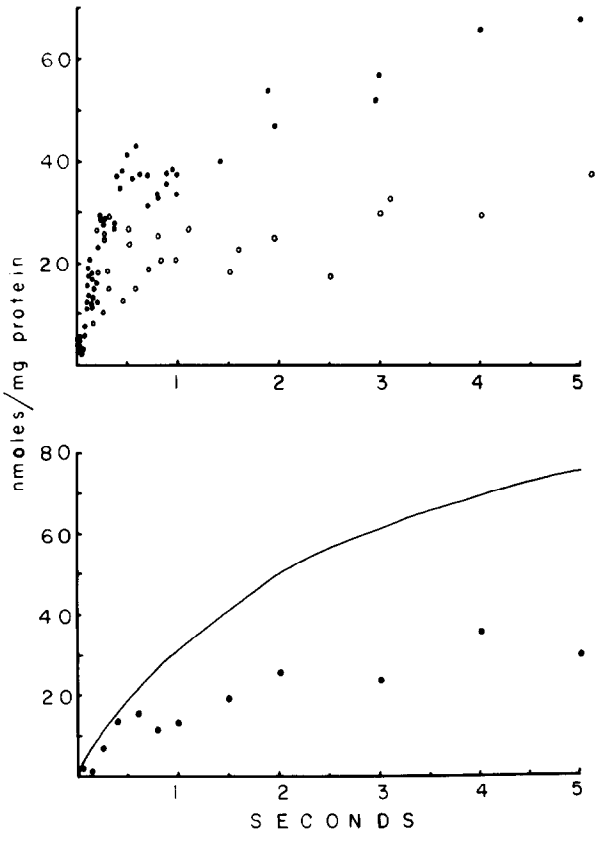

Fig.1. Top: P_i production in the presence (•) and in the absence (○) of Ca^{2+}. Reaction mixture: 20 mM MOPS (morpholinopropane sulphonic acid), 80 mM KCl, 5 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.5 mM [γ-^{32}P]ATP and 0.3–0.5 mg SR protein/ml. Ca^{2+}-independent activity was measured in the presence of 1.0 mM EGTA and no added calcium. Bottom: Ca^{2+}-uptake (solid line) and Ca^{2+}-dependent P_i 'extra' P_i production (•). The former activity was measured in the presence of 20 mM MOPS, 80 mM KCl, 10 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.1 mM murexide, 0.5 mM ATP and 0.8–1.0 mg SR protein/ml; the solid line in the graph was derived from an oscilloscope tracing representing light absorption changes undergone by the metallochromic indicator murexide. Ca^{2+}-dependent 'extra' P_i production (•) was calculated subtracting average values of actual data points obtained in the presence and in the absence of Ca^{2+}, as described above. In all experiments the pH was maintained at 6.8 and the temperature at 25°C.

Table 1
Levels of $E\sim P$ and magnitude of the initial P_i burst at various ATP concentrations

[ATP]	E P	P_i burst
0.010 mM	4.4	1.0
0.025 mM	4.4	3.0
0.10 mM	4.2	4.0
0.50 mM	3.3	18.0
1.00 mM	3.2	17.0

Figures refer to nmol/mg protein.

This scheme is compatible with the kinetic aspects of the experimental data. On the other hand, at saturating substrate concentrations, the magnitude of the initial P_i burst (table 1) is approximately three times greater than the number of available enzymatic sites (7 nmol/mg protein). On the contrary, the steady state levels of acid-stable phosphorylation ($E\sim P$) are always less than this number. It is then apparent that the proposed acid-labile phosphorylation ($E\cdot P$) occurs at sites other than, and independent of, the acid-stable phosphorylation ($E\sim P$). In fact, while $E\sim P$ formation is strictly Ca^{2+}-dependent, the P_i burst occurs even in the absence of Ca^{2+} (fig.1 and ref. [6]).

Unlike P_i production, ATP dependent Ca^{2+}-uptake by SR proceeds with a smooth time curve (fig.1, bottom) and is nearly completed in 10 s, reaching maximal values of 120–140 nmol/mg protein. Therefore Ca^{2+}-uptake is kinetically distinct from the initial burst and occurs in parallel with the P_i production, continuing after completion of the burst.

It was established with previous studies in the presence of oxalate that, in steady state condition, Ca^{2+}-transport is coupled with a 2:1 molar ratio to Ca^{2+}-dependent 'extra' ATP hydrolysis [7]. The 'extra' ATPase activity is defined as the difference between the total activity in the presence of Ca^{2+} and that in the absence of Ca^{2+}. With our experiments we have found that transport and enzyme activities are coupled with a 2:1 ratio even throughout the transient state. The importance of this finding becomes apparent if one considers the variable free energy requirement for active transport of one mole of calcium, in the presence of an initially low and thereafter increasing Ca^{2+}-gradient across the membrane. The constant molar ratio indicates a tight chemical coupling, compatible with a mechanism in which energy transduc-

tion occurs within a single enzyme unit. Such a tight coupling renders the ion pump least efficient in the initial phase and maximally efficient near the asymptote.

Acknowledgements

This work was partially supported by the National Institutes of Health (HL 16607) and the Muscular Dystrophy Association of America.

References

[1] Kanazawa, T., Yamada, S. and Tonomura, Y. (1971) J. Biochem. (Tokyo) 70, 95–123.
[2] Froehlich, J. P. and Taylor, E. W. (1975) J. Biol. Chem. 250, 2013–2021.
[3] Eletr, S. and Inesi, G. (1972) Biochim. Biophys. Acta 282, 174–179.
[4] Inesi, G. and Scarpa, A. (1972) Biochemistry 11, 356–359.
[5] Yamamoto, T. and Tonomura, Y. (1968) J. Biochem. 64, 137–145.
[6] Froehlich, J. P. and Taylor, E. W. (1976) J. Biol. Chem. 251, 2307–2315.
[7] Hasselbach, W. (1964) Prog. Biophys. Mol. Biol. 14, 167–222.