Biochimica et Biophysica Acta, 638 (1981) 341–344
Elsevier/North-Holland Biomedical Press

BBA 48189

PHOSPHOENZYME DECOMPOSITION IN SARCOPLASMIC RETICULUM ISOLATED FROM CAT CAUDOFEMORALIS, TIBIALIS AND SOLEUS

TAITZER WANG and ARNOLD SCHWARTZ

Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 45267 (U.S.A.)

(Received February 27th, 1981)

Key words: Phosphoenzyme decomposition; Ca^{2+}-ATPase; Sarcoplasmic reticulum; Muscle relaxation; (Cat skeletal muscle)

Decomposition of phosphoenzyme (E ~ P) in sarcoplasmic reticulum isolated from caudofemoralis, tibialis and soleus of cat hind leg skeletal muscles was studied under various conditions of monovalent cations. In the presence of Li^+, Na^+ and K^+ chosen for E ~ P formation and decomposition after quenching of E ~ P with EGTA, E ~ P in the caudofemoralis and tibialis sarcoplasmic reticulum decomposed faster than that in the soleus sarcoplasmic reticulum. Quenching the E ~ P formation with EGTA and ADP revealed that 30–40% of the total E ~ P formed in all types of sarcoplasmic reticulum was 'ADP sensitive'. Decomposition of the remaining E ~ P in caudofemoralis and tibialis sarcoplasmic reticulum was enhanced by ADP, which resulted in a multiphasic decomposition pattern. A larger portion of the remaining E ~ P in the soleus sarcoplasmic reticulum, on the other hand, decomposed in a monophasic manner and was not significantly influenced by ADP. The data on E ~ P decomposition clearly differentiate between the fast and slow muscle types.

Introduction

Muscle relaxation is caused by a decrease in the cytoplasmic concentration of Ca^{2+}, resulting from active transport of Ca^{2+} by sarcoplasmic reticulum [1–3]. It is important to understand why the rates of muscle relaxation of different muscle types are different. So far as the sarcoplasmic reticulum is concerned, the rapid relaxation associated with a fast muscle as compared with that of a slow muscle may be due to a larger amount (or a greater number of effective pump sites) of the sarcoplasmic reticulum Ca^{2+}-ATPase present in the fast muscle. In addition, it may also reflect differences in the intrinsic properties of the sarcoplasmic reticulum: the sarcoplasmic reticulum of the fast muscle may have higher Ca^{2+}-ATPase and Ca^{2+}-transport activities than those of a slow muscle.

This communication is concerned with the intrinsic properties of the sarcoplasmic Ca^{2+}-ATPase. The focus of the study is the decomposition of phosphoenzyme in sarcoplasmic reticulum from fast-twitch caudofemoralis and tibialis and slow-twitch soleus of the cat leg muscle. The advantages of these muscles are that they are homogeneous and that their twitch properties are well characterized [4]. The results are in accord with our recent findings that the decomposition of E ~ P in fast muscle sarcoplasmic reticulum is generally faster than that in slow muscle sarcoplasmic reticulum [5,6]. Similarities in the effects of ADP in E ~ P decomposition are observed among sarcoplasmic reticulum of fast-twitch cat caudofemoralis and tibialis and rabbit skeletal muscle [7] and between sarcoplasmic reticulum of slow-twitch cat soleus and dog cardiac muscle [7].

Methods

Materials. [γ-^{32}P]ATP (1 mCi in 0.03 μmol) was purchased from New England Nuclear. ADP (disodi-

um salt) was obtained from Sigma and ATP (disodium salt) from Boehringer Mannheim. All other chemical reagents were of analytical grade and stock solutions were prepared in distilled deionized water.

Preparation of sarcoplasmic reticulum vesicles. Our previously reported procedure for preparing sarcoplasmic reticulum vesicles from cat caudofemoralis, tibialis and soleus muscles [5] was followed with a slight modification [8]. The sarcoplasmic reticulum preparation was washed in a K^+-free buffer (30 mM Tris-maleate. pH 6.8) at 143 000 $\times g$ for 45 min and the isolated sarcoplasmic reticulum vesicles were suspended in the same buffer. Protein was determined by the biuret method, using bovine serum albumin (Sigma) as standard. All preparations were stored on ice and used within 20 h. The stability of the sarcoplasmic reticulum Ca^{2+}-ATPase prepared by this procedure was high; less than 8% of the activity was lost after 5 days at 0°C as shown by the E ~ P level measured at 116 ms. The yield of sarcoplasmic reticulum from each muscle was the same as that previously obtained [5].

Transient-state kinetic experiments. Rapid-mixing experiments were carried out using a chemical quench-flow apparatus and the procedure for isolation of the acid-stable E ~ P intermediate for ^{32}P counting was the same as previously described [9]. The concentration of free Ca^{2+} was calculated from an apparent EGTA-Ca^{2+} binding constant of $6.93 \cdot 10^5$ M^{-1} [10]. The vehicle solution used for the sarcoplasmic reticulum and substrate contained 3 mM $MgCl_2$, 5 mM NaN_3, and 20 mM Tris-maleate (pH 6.8). The temperature was maintained at 20°C.

E ~ P decomposition under various conditions of monovalent cations

(A) E ~ P formation in 20 mM choline chloride, Li^+, or Na^+ and decomposition in 80 mM K^+. The enzyme syringe contained 1 mg/ml caudofemoralis, tibialis or soleus sarcoplasmic reticulum, 30 mM choline chloride, LiCl, or NaCl, 100 μM EGTA, and 100 μM $CaCl_2$ (free [Ca^{2+}] 12 μM); the substrate syringe contained 20 μM ATP (including [γ-^{32}P]-ATP), 30 mM choline chloride, LiCl, or NaCl, 100 μM EGTA, and 100 μM $CaCl_2$; the EGTA syringe contained 6 mM EGTA and 240 mM KCl. All the above ligands were dissolved in the standard vehicle solution. The reaction of sarcoplasmic reticulum with ATP proceeded for 116 ms before quenching free Ca^{2+} with EGTA to initiate E ~ P decomposition. The time course of E ~ P decomposition was measured from 0 to 277 ms. A 9% perchloric acid solution including 1% sodium polyphosphate was used to quench the reaction. The entire sequence of reactions was carried out at 20°C.

(B) E ~ P formation in 100 mM Li^+ and decomposition in 67 mM Li^+ plus 33 mM Li^+ or Na^+. The procedures described above were used with the enzyme and substrate syringes containing 100 mM LiCl and the EGTA syringe containing 100 mM LiCl or NaCl.

(C) Effect of ADP. The experimental procedures were the same as described above for the E ~ P decomposition with the EGTA syringe containing 4 mM ADP. Formation of E ~ P was quenched with EGTA and ADP at 20.5 or 116 ms and the time courses of the subsequent E ~ P disappearance were measured from 0 to 277 ms at 20°C.

Results

The rate constants of initial E ~ P decompositions under various conditions of monovalent cations and ADP are summarized in Table I.

The decompositions of soleus E ~ P are clearly slower than those of caudofemoralis and tibialis E ~ P under identical conditions. When E ~ P is formed in 30 mM choline, Li^+, or Na^+ for 116 ms and

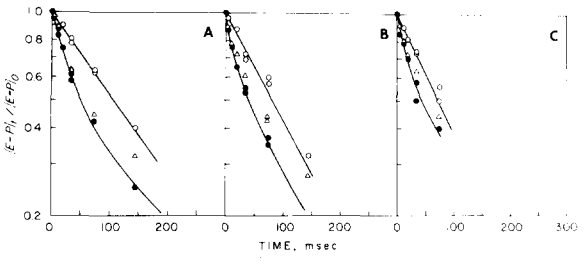

Fig. 1. Semilogarithmic plots of E~P decomposition with respect to time: caudofemoralis, ●———●; tibialis, △———△, and soleus, ○———○. E~P was formed at 20°C in 20 mM Tris-maleate buffer (pH 6.8) containing 10 μM ATP, 12 μM Ca^{2+}, 3 mM Mg^{2+}, 5 mM NaN_3, and 30 mM choline chloride, Li^+, or Na^+. At 116 ms, buffer containing 3 mM Mg^{2+}, 5 mM NaN_3, 6 mM EGTA, and 240 mM K^+ was added to initiate E~P decomposition. Final concentrations of the monovalent cations in the decomposition medium: A, 20 mM choline chloride and 80 mM K^+; B, 20 mM Li^+ and 80 mM K^+; and C, 25 mM Na^+ and 80 mM K^+.

TABLE I

RATE CONSTANT OF INITIAL E ~ P DECOMPOSITION UNDER VARIOUS CONDITIONS OF MONOVALENT CATIONS AND ADP

Ligand condition [a]		k_d (s^{-1}) [b]		
E ~ P formation	E ~ P decomposition	Caudofemoralis	Tibialis	Soleus
Choline chloride	Choline chloride + K^+	20–30	20–30	6.5
Li^+	$Li^+ + K^+$	20–30	20–30	8.5
Na^+	$Na^+ + K^+$	20–30	20–30	9.7
Li^+	Li^+	–	4.1 [c]	1.8 [d]
Li^+	$Li^+ + Na^+$	–	5.9	3.6
K^+	K^+ + ADP	40–50	40–50	11.0
Choline chloride	Choline chloride + ADP	23	19	1.5

[a] See text for ligand concentration.
[b] Estimates of hand-drawn slopes.
[c] Followed by a brief, slow decomposition.
[d] Followed by a 30 ms induction period.

decomposed (after quenching with EGTA) in the respective cation solution plus 80 mM K^+, a monophasic decomposition accounting for more than 60% of total E ~ P is observed for the soleus E ~ P, whereas, in the same time range, a biphasic decomposition is apparent for both caudofemoralis and tibialis E ~ P (Fig. 1). When the E ~ P is formed and decomposed in 100 mM Li^+, an induction period of 30 ms preceding a monophasic decomposition is observed for the soleus E ~ P, whereas for caudofemoralis and tibialis E ~ P, there is a very slow phase of E ~ P decomposition (data not shown).

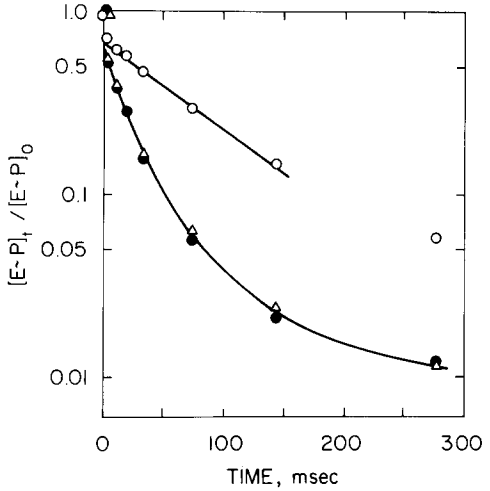

Fig. 2. Semilogarithmic plots of E~P decay with respect to time: effect of ADP in the presence of K^+. E~P was formed at 20°C in 20 mM Tris-maleate buffer (pH 6.8) containing 10 μM ATP, 12 μM Ca^{2+}, 3 mM Mg^{2+}, 5 mM NaN_3, and 100 mM K^+. At 116 ms, buffer containing 3 mM Mg^{2+}, 5 mM NaN_3, 100 mM K^+, 6 mM EGTA, and 4 mM ADP was added to initiate E~P decay. Final concentration of ADP was 1.33 mM. Cat caudofemoralis, ●———●; tibialis, △———△; soleus, ○———○.

In the presence of 1.33 mM ADP and 100 mM K^+, 30–40% of the total E ~ P from all types of sarcoplasmic reticulum interacts rapidly with ADP. The decomposition of the remaining E ~ P in the caudofemoralis and tibialis is enhanced by ADP, but that of the soleus E ~ P is not affected (Fig. 2). Similar effects of ADP are observed in 100 mM choline chloride, except that the overall rate of E ~ P decomposition in each type of sarcoplasmic reticulum is smaller than those measured in the presence of K^+.

Discussion

The three types of cat skeletal muscle sarcoplasmic reticulum share the common properties of rabbit skeletal and dog cardiac sarcoplasmic reticulum with respect to the relative sensitivity of E ~ P toward Li^+, Na^+, and K^+ at 0°C [11–14]. A much greater enhancement of E ~ P decomposition by K^+ when the E ~ P is formed in a non-K^+ medium (choline, Li^+, or Na^+) is also in accord with that observed for

rabbit skeletal sarcoplasmic reticulum [15].

The present communication emphasizes especially the correlation between the rate of E ~ P decomposition observed under various conditions of monovalent cations and ADP and the reported twitch characteristics of the muscle [4] from which the sarcoplasmic reticulum is isolated. As clearly shown, the initial decomposition of E ~ P in caudofemoralis and tibialis sarcoplasmic reticulum (the muscles have isometric half-relaxation times of 15 and 15–20 ms, respectively [4]) are generally faster than that of the soleus muscle sarcoplasmic reticulum (half-relaxation time 45 ms [4]). A slower rate of decomposition of tibialis E ~ P as compared with caudofemoralis E ~ P is observed only after 50 ms of decomposition. When the decomposition experiment is carried out in the presence of ADP, an instantaneous decrease in E ~ P level is observed, indicating the existence of an 'ADP-sensitive' phosphoenzyme that reacts rapidly with ADP, presumably to yield ATP [1–3,16,17]. In these cases the difference between the sarcoplasmic reticulum from fast and slow muscles is revealed in the decomposition pattern of the remaining E ~ P. In caudofemoralis and tibialis sarcoplasmic reticulum the E ~ P decomposition pattern is multiphasic whereas for the soleus sarcoplasmic reticulum it is monophasic. Although the detailed mechanism in both cases is unknown, it is clear that ADP enhances the decomposition of E ~ P in caudofemoralis and tibialis sarcoplasmic reticulum more effectively than in the soleus sarcoplasmic reticulum, similar to what we have observed for skeletal and dog cardiac sarcoplasmic reticulum [7].

The clear similarities among sarcoplasmic reticulum from cat caudofemoralis and tibialis and rabbit skeletal muscles and between sarcoplasmic reticulum from cat soleus and dog cardiac muscles indicate that there are differences in kinetic properties between sarcoplasmic reticulum Ca^{2+}-ATPase isolated from fast- and slow-twitch muscles. In addition to the pump site density [18] that may be higher for the fast muscle sarcoplasmic reticulum than for the slow muscle sarcoplasmic reticulum, the intrinsic rate constants of rate-determining steps [5–7,9] in the transient phase of ATP hydrolysis may also differentiate between muscle types. The results provide a strong basis for studying the factors causing the differences in the intrinsic properties between fast and slow muscle sarcoplasmic reticulum.

Acknowledgments

We thank Ms. L.I. Tsai for her skillful preparation of cat muscle sarcoplasmic reticulum. This work was supported by PHS Grants P01 Hl 22619-03 (IV-A) and Univeristy of Cincinnati Research Council Award.

References

1 Hasselbach, W. (1979) Top. Curr. Chem. 78, 1–56
2 Inesi, G. (1979) in Membrane Transport in Biology (Giebisch, G., Tosteson, D.C., and Ussing, H.H., eds.), pp. 357–393, Springer-Verlag, Berlin
3 Tada, M., Yamamoto, T. and Tonomura, Y. (1978) Physiol. Rev. 5, 1–79
4 Van Winkle, W.B., Entman, M.L., Bornet, E.P. and Schwartz, A. (1978) J. Cell. Physiol. 97, 121–135
5 Wang, T., Grassi de Gende, A.O. and Schwartz, A. (1979) J. Biol. Chem. 254, 10675–10678
6 Sumida, M., Wang, T., Schwartz, A., Younkin, C. and Froehlich, J.P. (1980) J. Biol. Chem. 255, 1497–1503
7 Wang, T., Grassi de Gende, A.O., Tsai, L.I. and Schwartz, A. (1981) Biochim. Biophys. Acta 637, 523–529
8 Wang, T., Tsai. L.I., Solaro, R.J., Grassi de Gende, A.O. and Schwartz, A. (1979) Biochem. Biophys. Res. Commun. 91, 356–361
9 Sumida, M., Wang, T., Mandel, F., Froehlich, J.P. and Schwartz, A. (1978) J. Biol. Chem. 253, 8772–8777
10 Harafuji, H. and Ogawa, Y. (1980) J. Biochem. (Tokyo) 87, 1305–1312
11 Ribeiro, J.M.C. and Vianna, A.L. (1978) J. Biol. Chem. 253, 3153–3157
12 Duggan, P.F. (1977) J. Biol. Chem. 252, 1620–1627
13 Jones, L.R., Besch, H.R., Jr. and Watanabe, A.M. (1977) J. Biol. Chem. 252, 3315–3323
14 Jones, L.R., Besch, H.R., Jr and Watanabe, A.M. (1978) J. Biol. Chem. 253, 1643–1653
15 Shigekawa, M. and Dougherty, J.P. (1978) J. Biol. Chem. 253, 1451–1458
16 Kanazawa, T., Yamada, S., Yamamoto, T. and Tonomura, Y. (1971) J. Biochem. (Tokyo) 70, 95–123
17 Martonosi, A., Lagminska, E. and Oliver, M. (1974) Ann. N.Y. Acad. Sci. 227, 549–567
18 Shigekawa, M., Finegan, J.-A.M. and Katz, A.M. (1976) J. Biol. Chem. 251, 6894–6900