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Time-Resolved Partial Reactions of the SR Ca-ATPase Investigated with a Fluorescent Styryl Dye

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The SR Ca-ATPase pumps two Ca^{2+} per ATP hydrolyzed into the lumen of the SR in exchange for two H^+. So far, only fluorescent styryl dyes may be used to detect electrogenic ion movements into and out of the binding sites of the pump.[1,2] Time-resolved kinetics can be analyzed by ATP-concentration jump experiments in which ATP is released from its inactive precursor caged-ATP by fast UV-laser flashes.[3] After release of ATP, a rising and a falling fluorescence intensity could be observed. The pump proceeds through its pump cycle and, as function of pH, it reaches a new steady state in a more or less protonated $P\text{-}E_2$ form (FIG. 1).

The time constant of the rising phase was 35 ± 5 ms. This constant was independent of substrate concentrations when Ca^{2+} > 100 nM (Ca^{2+} binding not limiting), when pH < 8 (at pH > 8, the ATP-release reaction is limiting; see FIG. 1), and when ATP > 20 μM (ATP binding not limiting).

The time constant of the falling phase had a minimum of 1.38 ± 0.29 s at pH 7, independent of the Ca^{2+} concentration. At higher pH, the H^+ concentration becomes a limiting factor and, at pH < 6.5, the affinity of the protein for H^+ is decreased.

The Arrhenius plots of the time constants of both fluorescence phases showed activation energies of 80–90 kJ/mol, comparable to the activation energy of $E_1\text{-}E_2$ transitions in the Na,K-ATPase. Therefore, the rate-limiting step of the rising phase is assumed to be a conformation transition included in the following scheme:[4]

$$(Ca_2)E_1\text{-}P \rightarrow P\text{-}E_2Ca_2 \rightarrow P\text{-}E_2{}^*Ca \rightarrow P\text{-}E_2Ca \rightarrow P\text{-}E_2.$$

On the basis of the very slow falling phase of the fluorescence, which reflects H^+ binding, the reaction sequence from the $P\text{-}E_2$ state onwards has to be supplemented by an additional step, assumed to be a slow conformational relaxation that precedes H^+ binding, such as $\cdots \rightarrow P\text{-}E_2{}^*s \rightarrow P\text{-}E_2 \rightarrow P\text{-}E_2H_2$.

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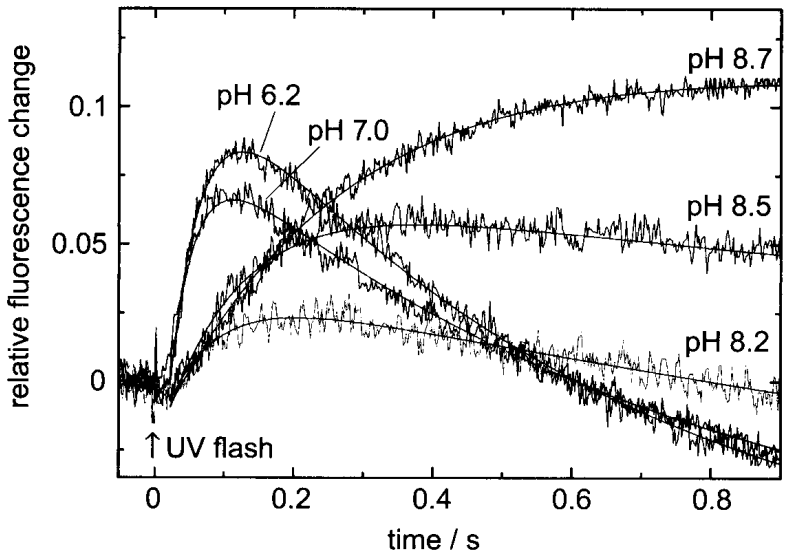

FIGURE 1. Fluorescence changes induced by the release of about 20 μM ATP. The UV flash occurred at time $t = 0$. The buffer contained 25 mM tricine, 50 mM KCl, 1 mM $MgCl_2$, 650 nM styryl dye 2BITC,[2] and 18 μg of SR Ca-ATPase. pH was adjusted according to the respective labels. The time course of the fluorescence was fitted with the sum of two exponentials. The constant level of fluorescence after times longer than 1 s was dependent on the steady state distribution into which the ion pumps relaxed after the ATP-concentration jump and was controlled by the amount of H^+ ions bound in the new steady state.

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