Biochimica et Biophysica Acta, 778 (1984) 615–617
Elsevier

BBA Report

BBA 70194

PRESERVATION OF FUNCTIONAL INTEGRITY DURING LONG TERM STORAGE OF A BIOLOGICAL MEMBRANE

ROBERT MOURADIAN, CHRISTOPHER WOMERSLEY, LOIS M. CROWE * and JOHN H. CROWE

Department of Zoology, University of California, Davis, CA 95616 (U.S.A.)

(Received April 11th, 1984)
(Revised manuscript received September 17th, 1984)

Key words: Membrane storage; Trehalose; Ca^{2+} transport; ATPase; (Sarcoplasmic reticulum)

Sarcoplasmic reticulum vesicles freeze-dried in the presence of trehalose retain most of their original biological activity for short periods. When the dry vesicles are stored for longer periods in air, Ca^{2+}-transport becomes uncoupled from ATPase activity within a few days. However, when they are stored under vacuum, ATPase activity, Ca^{2+} transport, and coupling between Ca^{2+} transport and ATP utilization are maintained essentially intact for at least 110 days.

We have recently shown that isolated vesicles of sarcoplasmic reticulum can be stabilized at low water activities if they are dried in the presence of certain carbohydrates, the most effective of which is trehalose [1]. Upon rehydraton, the dry membrane-trehalose preparations yield vesicles which are similar structurally and functionally to freshly prepared ones [2]. Even more recently, we have provided evidence concerning the physical basis for the remarkable ability of trehalose to preserve these dry membranes [3–6]. These previous results were obtained with membranes stored in the dry state for at most a few days. However, it is likely that degradative reactions occur with longer term storage which violate the structural and functional integrity of the membranes. In fact, we have shown elsewhere that degradative oxidations lead to loss of all biological activity within a few weeks, even when the membranes are stored in high concentrations of trehalose [7]. In this brief report we demonstrate that when they are stored under vacuum biological activity is retained at levels similar to those of freshly prepared vesicles for at least 110 days.

Sarcoplasmic reticulum vesicles were prepared from lobster muscle, as previously described [8]. Protein content of the preparation was determined by the Coomassie blue method or the method of Lowry et al. [9], using bovine serum albumin as the standard. Vesicles were diluted to a concentration of 5 to 10 mg/ml and trehalose was then added as concentrated solutions, after which 0.5–1-ml aliquots were frozen in liquid nitrogen and lyophilized. Immediately following lyophilization, half the samples were transferred to Pyrex tubes which had been previously sealed at one end. The tubes were then evacuated and sealed. Remaining samples were stored open to the atmosphere in the same tubes in which they were lyophilized. These air stored samples were stored over $CaSO_4$ desiccant. At intervals, samples were rehydrated to their original volume with deionized water and suspended by several strokes in a glass tissue homogenizer. The protein concentration of the rehydrated sample was again determined by the Coomassie blue method, and the carbohydrate content was assayed by the anthrone method [10]

* To whom correspondence should be addressed.

or by high performance liquid chromatography. Following rehydration, samples were assayed for ATPase activity, calcium transport activity, and coupling ratio (moles calcium transported/mole ATP hydrolyzed) according to the methods of Martonosi and Feretos [11]. Calcium transport activity was measured as rate of uptake of $^{45}Ca^{2+}$ by sarcoplasmic reticulum vesicles [12].

Previous results have shown that when sarcoplasmic reticulum vesicles are lyophilized with less than 0.3 g trehalose/g membrane, rehydration typically produces vesicles with a large decrease in coupling ratio and calcium uptake and a concurrent increase in the rate of ATP hydrolysis [2]. The loss in biological activity was correlated with various structural changes such as phase transitions, displacement of membrane proteins from their normal position in the membrane, and vesicle-vesicle fusions [3]. The present results indicate that changes in biological activity similar to those occurring on lyophilization can occur during storage of the vesicles in air; rapid uncoupling and an increase in the ATPase activity occur even in the presence of high levels of trehalose (0.5 g/g) in the sample (Fig. 1). After only 10 days storage in air Ca^{2+} uptake declined to $< 50\%$ of the original, and ATPase activity increased more than 3-fold. Both these effects are reflected in the decline of coupling between ATP utilization and Ca^{2+} uptake over the same time period (Fig. 1). The loss of coupling and ability to accumulate Ca^{2+} and increased ATPase activity are characteristic of 'leaky' microsomes which cannot retain transported Ca^{2+} [13]. A likely cause of this damage is oxidation of membrane lipids (cf. Refs. 13 and 14); in keeping with this suggestion is the fact that the hydrocarbon chains of phospholipids in the sarcoplasmic reticulum membranes used here are highly unsaturated and thus would be expected to be subject to oxidation damage, leading to increased permeability to Ca^{2+}, and subsequently to oxidative damage to the ATPase. In fact, we have shown elsewhere that such oxidations do occur in these membranes and have explored the mechanism by which they damage the membranes [7]. The possibility that these oxidations are responsible for loss of biological activity of the membranes stored in air was tested by storing the dry membranes under vacuum, with the results shown in Fig. 1. When

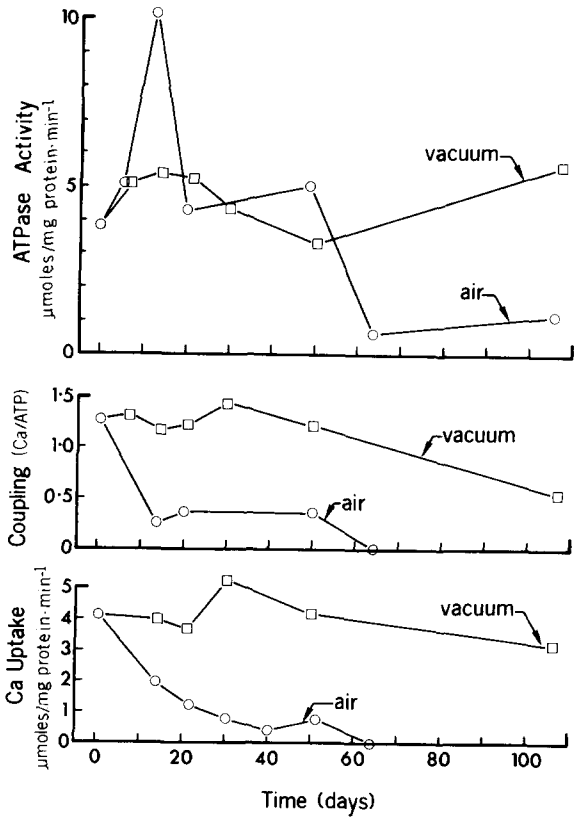

Fig. 1. ATPase activity (top), coupling ratio (middle) and Ca^{2+} uptake activity (bottom) in rehydrated sarcoplasmic reticulum vesicles previously stored in vacuum or in air for the indicated intervals. Samples contained 0.5 g trehalose/g membrane. All the activities in freshly lyophilized vesicles are at least 80% of those in freshly prepared vesicles [2].

vesicles dried with 0.5 g trehalose/g membrane were stored under vacuum, activities were preserved efficiently, with Ca^{2+} transport activity at least 90% of the original after 110 days (Fig. 1). ATPase activity increased slightly over the first two weeks of storage and then slowly declined until about 50 days. Beyond 50 days a rise in ATPase activity which coincided with more extensive uncoupling was recorded. Despite this apparent uncoupling after long term storage, Ca^{2+} uptake was undiminished, possibly due to the increased ATPase activity. All parameters were highly significantly different from samples stored in air (Fig. 1).

We conclude that under appropriate conditions sarcoplasmic reticulum membranes may be stored

dry for at least 110 days without serious loss of biological activity. In order for this preservation to be achieved, the membranes were lyophilized in the presence of trehalose and stored dry and under vacuum. We emphasize that the most efficient carbohydrate available for stabilizing the dry membranes is trehalose [1]. Furthermore, we expect the results presented here to be of considerable practical significance in preservation of membranes.

We gratefully acknowledge support of the National Science Foundation, U.S. through grants PCM 80-04720 and PCM 82-17538 and National Sea Grant through grant RA/47.

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