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Biophysical Research Group,
Institute of Physics,
The State University,
Utrecht (The Netherlands)

C. BRIL
D. J. VAN DER HORST
S. R. POORT
J. B. THOMAS

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Stabilization of cardiac microsomal calcium uptake by hypertonic sucrose solutions

During storage in isotonic sucrose cardiac microsomes rapidly lose their ability to take up calcium, and most investigators have found almost complete loss of this activity after only a few days[1–4]. Sucrose density gradients have been used to obtain cardiac microsomes of greater stability, which has been attributed to removal of contaminants on the density gradients[5,6]. This interpretation may be incorrect, however, because the ability to take up calcium can be preserved simply by storage of the microsomes in hypertonic sucrose solutions such as those in which microsomes were recovered from the sucrose density gradients used in earlier studies.

Microsomes were prepared from canine ventricles by a slight modification of the standard method of this laboratory[6]. The muscle, obtained from dogs anaesthetized with pentobarbital, was homogenized for 60 sec at top speed in a Waring blender in 3–4 vol. of ice cold 10 % sucrose (w/v) containing 6 mM $MgCl_2$, and buffered with 20 mM Tris chloride at pH 8.0. After centrifugation for 5 min at 800 × *g*, the supernatant was filtered through 6 layers of gauze and centrifuged for 30 min at 15000 × *g*. The supernatant was again filtered through gauze and centrifuged for 60 min at

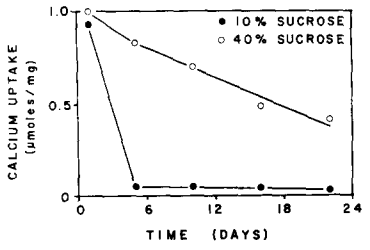
47000 × *g*. The microsomal pellets were taken up in various solutions (see below) and homogenized by 10 gentle strokes in a glass Potter–Elvehjem homogenizer fitted with a teflon pestle.

Calcium uptake was measured in solutions buffered at 10^{-5} M free Ca^{2+} by 0.125 mM ethyleneglycol-bis-(β-aminoethylether)-N,N'-tetraacetic acid (calcium-45 salt) and 0.015 mM ethyleneglycol-bis-(β-aminoethylether)-N,N'-tetraacetic acid. The binding constants used were obtained from CHABEREK AND MARTELL[7]. The reaction mixture contained 0.025 mg/ml microsomal protein, 0.12 M NaCl, 2.5 mM Tris oxalate, 5.0 mM MgATP and 10 mM histidine at pH 6.6, and contained approx. 0.01 μC ^{45}Ca per ml. Microsomes were removed by filtration through Ha (0.45 mμ) Millipore filters and the radioactivity in the filtrate was determined in a Packard Tri-Carb liquid scintillation counter as described earlier[6]. Reactions were run at 25°.

Microsomes kept on ice in 10% sucrose rapidly lost the ability to take up calcium, whereas an aliquot of the same preparation stored in 40% sucrose retained more than half the initial activity after 2 weeks (Fig. 1).

Stabilization of microsomal calcium uptake by hypertonic sucrose was also seen when the microsomes were exposed to urea or LiBr. In these experiments, cardiac microsomes were suspended in 2 mM EDTA, 0.65 mM dithiothreitol and dilute Tris at pH 7.0, and kept on ice overnight in 0.8 M urea or 1.0 M LiBr in various concentrations of sucrose. Calcium uptake was measured subsequently in reaction mixtures in which the urea and LiBr were diluted to much lower concentrations. Exposure to either urea or LiBr in isotonic sucrose greatly reduced the ability of the microsomes to sequester calcium, but the detrimental effects of both reagents were considerably reduced when exposure was in the presence of hypertonic sucrose (Fig. 2). In other experiments the calcium uptake of microsomes exposed overnight to 1.0 M LiBr in 40% sucrose was measured after the microsomes had been washed free of the salt. Under these conditions, calcium uptake was fully restored to control level.

It is apparent that loss of the ability to take up calcium, due both to the detrimental effects of aging and to the denaturing effects of urea and LiBr, is reduced when cardiac microsomes are suspended in hypertonic sucrose solutions. Isotonic



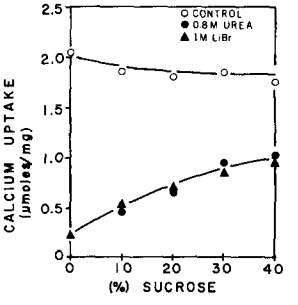


Fig. 1. Effects of hypertonic sucrose on stability of cardiac microsomal calcium uptake. Calcium uptakes of microsomes stored in 10% sucrose (●) or in 40% sucrose (○) were measured after dilution of the storage solutions.

Fig. 2. Effects of sucrose on stability of cardiac microsomes in urea and LiBr. Cardiac microsomes, prepared in solutions containing 0.65 mM dithiotreitol and 2 mM EDTA, were exposed to 0.8 M urea (●) or 1.0 M LiBr (▲) as described in the text. Calcium uptakes were measured after dilution of the urea to 0.020 M and LiBr to 0.013 M, and compared to a control microsomal preparation (○).

sucrose solutions have long been known to provide excellent media for preparation of subcellular particles, but hypertonic sucrose often has deleterious effects on such particles. Although hypertonic sucrose solutions preserve mitochondrial morphology[8], sucrose concentrations greater than isotonic impair both oxygen uptake[9] and oxidative phosphorylation[10] of isolated mitochondria. Similarly, nuclear preparations exposed to hypertonic sucrose solutions lose their ability to incorporate amino acids[11] and to incorporate [^{14}C]orotic acid into the pyrimidines of their ribonucleic acids[12]. On the other hand the present finding cannot be considered totally unexpected because sucrose has the general property of stabilizing the conformations of a variety of macromolecules[13]. In the field of muscle biochemistry, for example, it is well established that G-actin free of nucleotides and divalent cations is best prepared in approx. 50 % sucrose[14]. F. N. BRIGGS (personal communication), who independently observed the stabilization of cardiac microsomal calcium uptake by storage of the microsomes in hypertonic sucrose, attributed this stabilizing effect to inhibition of a lysosomal contaminant in these microsomes (which has also been found in our laboratory by Dr. J. O. Finney). The present results suggest that hypertonic sucrose, by preventing the structure-disrupting actions of LiBr and urea, has additional effects to stabilize these microsomes.

The use of hypertonic sucrose is clearly of value in the preparation and study of cardiac microsomes. Not only is the storage of these preparations improved under such conditions, but agents potentially useful in fractionating this heterogeneous material can be used without destroying important biochemical activities.

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Department of Medicine and Physiology,
The University of Chicago,
Chicago, Ill. 60637 (U.S.A.)

DORIS I. REPKE
ARNOLD M. KATZ

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