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The Mitochondria as Biosensors for the Monitoring of Detergent Compounds in Solution

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The monitoring of detergents in surface waters is a problem of great environmental concern as a consequence of their steadily increasing use worldwide. Therefore, many analytical procedures have been proposed for monitoring detergent compounds in solution (1–5).

The toxicological consequences of the increase of detergent compounds in aquatic environment have been widely studied in whole animals. At the same time, *in vitro* experiments in cells and subcellular structures have been performed to establish the molecular causes which are responsible for the toxic effects in animals. In this regard, mitochondria are the preferred target for many toxic compounds, since damage to mitochondria, which synthesize ATP for the cell, will cause damage to the cell as a whole.

For this reason mitochondria have been largely used as biosensors (6–10) and in the present paper we propose that mitochondria from rat liver be used as biosensors for monitoring of various kinds of detergent compounds in solution.

Materials and methods. Mitochondria from rat liver and beef heart were prepared following the usual procedures (8, 11). The protein concentration was determined by the Lowry method (12). Beef heart mitochondria after preparation were either freshly used or stored at −20°C in a freezer. Swelling experiments were followed with a Jenway 6400 spectrophotometer under stirring and at room temperature. After the addition of medium (2.5 ml) and mitochondria the spectrophotometer was calibrated at zero absorbance at 540 nm. Therefore the decrease in absorbance due to addition of detergents appears as negative absorbance. In all experiments the medium composition was 0.25 M sucrose, 10 mM Tris, pH 7.4, 0.5 mM EDTA.

All reagents were of analytical grade. Detergent compounds (SDS,[2] sodium dodecyl sulfate; Triton, t-octylphenoxypolyethoxyethanol; CTA, dodecyltrimethylammonium bromide) were supplied by Sigma (Milan, Italy). Nonylphenol polyethoxylate (NPOEO) and linear alkylbenzene sulfonate (LAS; fw 316) were a kind gift from Dr. A. Orio.

Results and discussion. Detergent compounds are subdivided in three categories, cationic, anionic, and neutral, all having a long aliphatic, hydrophobic chain in common. This hydrophobic chain interacts with the lipidic bilayer of the mitochondrial membrane. The mitochondrial membrane is not permeable to sucrose, but the interaction with detergent compounds alters the membrane permeability and sucrose enters the mitochondrial matrix. Sucrose entrance, by a colloid-osmotic mechanism, gives rise to swelling (13). The

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[2] Abbreviations used: SDS, sodium dodecyl sulfate; Triton, t-octylphenoxypolyethoxyethanol; CTA, dodecyltrimethylammonium bromide; LAS, linear alkylbenzene sulfonate; NPOEO, nonylphenol polyethoxylate.

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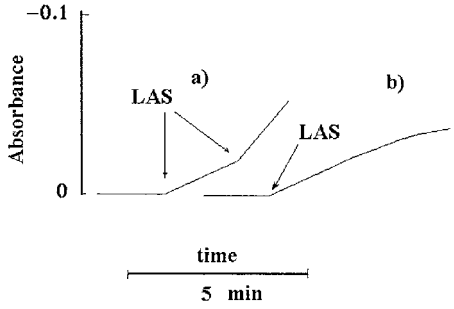

FIG. 1. LAS induces swelling in nonenergized mitochondria. Medium composition: 0.25 M sucrose, 10 mM Tris, pH 7.4, 0.5 mM EDTA. To the incubating medium (2.5 ml), (a) rat liver mitochondria were added and the final mitochondrial concentration was 0.5 mg/ml. After resetting the spectrophotometer at zero absorbance, 30 and 20 μM LAS in two successive additions were added. The swelling rate is the slope of absorbance change against time. In b, 0.5 mg/ml (final concentration) of thawed beef heart mitochondria was added to the incubating medium. After resetting the spectrophotometer at zero absorbance, 30 μM LAS was added.

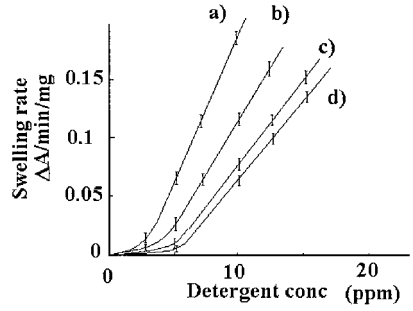

FIG. 3. Rates of absorbance change with different detergent compounds: SDS (a), CTA (b), Triton (c), and NPOEO (d). Medium composition as in Fig. 1. Mitochondrial concentration (rat liver) 0.125 mg/ml. Each point is the average from five replicates. The concentrations are reported in ppm. In the case of CTA, 1 ppm corresponds to 3.2 μM. In the case of SDS, 1 ppm is 3.4 μM.

swelling induces an absorbance change (decrease) at 540 nm (13). Therefore, mitochondria resuspended in a sucrose medium allow the spectrophotometric monitoring of detergent compounds in solution. Figure 1 shows this behavior when LAS is the detergent inducing mitochondrial swelling. Figure 1a also shows that the rate of decreasing absorbance (the slope of ΔA against time) increases with the detergent concentration. This property allows the monitoring of LAS concentration in solution as discussed below.

Since the swelling is due to the interaction between detergent and lipidic bilayer, this interaction, and consequently the sensitivity of the method, will depend on the mitochondria/detergent ratio. The data reported in Fig. 2 (the rate of absorbance change at different mitochondrial concentrations) actually show that this hypothesis is correct, since by decreasing the protein (i.e., the mitochondrial) concentration the absorbance change rate and, consequently, the sensitivity increase. The results reported in Fig. 2 suggest that the most favorable conditions (i.e., protein concentration) are in the range of 0.125 mg/ml. Under these experimental conditions we have measured the rates of absorbance change as a function of the detergent concentration with many detergent compounds. The results are reported in Fig. 3. For each detergent the lowest dose which can be monitored is obtained by extrapolation of the straight line at $\Delta A = 0$, as shown by the dotted line.

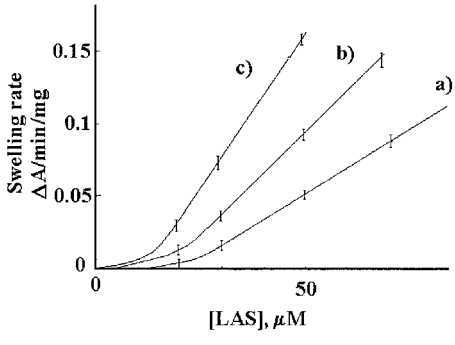

FIG. 2. The swelling rate depends on the mitochondrial protein concentration. Medium composition as in Fig. 1. The swelling rates induced by LAS in different rat liver mitochondrial concentrations are reported: (a) 0.5, (b) 0.25, and (c) 0.125 mg/ml. The lower limit is roughly estimated by extrapolating the linear portion to $\Delta A/\text{min} = 0$. Each point is the average from five replicates.

As cited in the literature, other chemical and physical methods have been proposed for monitoring detergent compounds (1–5), but the procedures are selective for individual groups of surfactants, are sensitive to the presence of interfering compounds in solution (see below), and require solvent extraction. A single procedure for monitoring all detergent compounds based on the use of a biosensor has never been proposed.

By means of this procedure it is possible to monitor each detergent, but, when several detergent compounds are simultaneously present in solution, the procedure does not distinguish between the contributions of each detergent to the observed absorbance change. In this respect, however, it has been demonstrated that cationic and anionic detergents are captured by energized mitochondria and lysosomes, respectively (14, 15). Therefore this property can be used to separate under appropriate conditions (14, 15) the cationic detergents from a mixture of detergents by addition of energized mitochondria and subsequent centrifugation. Analogously, the negatively charged detergents can be separated from the solution by addition of energized lysosomes and subsequent centrifugation or fast filtration. In practice, the procedure is made easier by the fact that liver mitochondria are obtained from the pellet and the lysosomes are obtained from the supernatant in the same centrifugation (16).

For the monitoring procedure described here beef heart mitochondria instead of rat liver mitochondria can be used (Fig. 1b). The mitochondria from beef heart offer the advantage that once prepared, they can be stored in a freezer for months (8). Once they are thawed and resuspended in the sucrose medium, the responses are the same as those with freshly prepared rat liver mitochondria. The only difference is that after some minutes the linearity of the response, ΔA against time, is no longer conserved (Fig. 1b); however, if the initial slope is measured, the results and the sensitivity are the same. This is a significant advantage since beef heart mitochondria can be stored once prepared, and beef heart tissue is readily available.

Interference could be due to the presence of chemical compounds (i.e., organometallic compounds) which induce the opening of a transition pore with consequent swelling even under nonenergized conditions (17). In this case, however, by operating in the presence of Cyclosporine, the inhibitor of the permeant pore, the interferences are excluded. Other interferences could be due to the presence of divalent ions such as Ca^{2+} or Mg^{2+} since these compounds alter the membrane structure. The problem is avoided by working in the presence of EDTA in the medium. Organic compounds such as alcohols give interferences only at high concentrations, above millimolar.

Conclusions. A procedure which allows the selective monitoring of detergents in solution has been described. The method utilizes the sensitivity of rat liver mitochondria (or beef heart mitochondria, freshly prepared or thawed) toward detergent compounds since these compounds give rise to mitochondrial swelling. Swelling is measured by an absorbance change and the rate of this change is proportional to the detergent concentration. Since a biosensor for the monitoring of all surfactants (cationic, anionic, and neutral) has not yet been proposed, and since the procedure is not greatly influenced by the presence of interfering compounds, this method is proposed as a prescreening test for direct monitoring of all kinds of surfactants in environmental samples.

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A Study of His-Tagged Alkaline Phosphatase Immobilization on a Nanoporous Nickel–Titanium Dioxide Film

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Immobilization is an important step in the development of both biosensors and binding assays, but traditional immobilization methods have disadvantages in terms of retention of bioactivity as well as limited

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