REVISTA ESPAÑOLA DE FISIOLOGIA, 36, 83-88, 1980

Solubilization of Inner Mitochondrial Membranes by Triton X-100. Effect of Ionic Strength and Temperature

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(Received on August 6, 1979)

J. I. G. GURTUBAY. *Solubilization of Inner Mitochondrial Membranes by Triton X-100. Effect of Ionic Strength and Temperature.* Rev. esp. Fisiol., 36, 83-88. 1980.

Rat liver inner mitochondrial membranes have been subjected to the solubilizing action of the non-ionic detergent Triton X-100 under a variety of ionic strength and temperature conditions. Increasing ionic strength has little influence on the amount of solubilized membrane protein and lipid phosphorus. Calcium chloride actually increases the proportion of solubilized protein. This effect is preserved by 1 mM EDTA. Increasing temperatures tend to decrease the proportion of protein solubilized by the detergent. SDS-polyacrylamide gel electrophoresis fails to reveal any difference in polypeptide composition of the membrane fraction solubilized under the various conditions. However, differences are observed in the solubilization of individual cytochromes. The data are interpreted in terms of changes in membrane architecture induced by the various conditions of the incubation medium.

Non-ionic detergents, such as Triton X-100 {α-[4-(1,1,3,3-tetramethylbutyl)-phenyl]-ω-hydroxy-poly (oxy-1,2-ethanediyl)} have found considerable application in the field of membrane solubilization, because of their high solubilizing power, with little perturbing effects on the structure and catalytic activity of proteins.

According to our experience, Triton X-100 is an effective and mild solubilizing agent of mitochondrial (5, 6) and other (9) membranes. Its main disadvantage is the low critical micellar concentration, which makes difficult the detergent removal by dialysis. Nevertheless, this inconvenience can be easily overcome by the use of detergent-adsorbing resins (8).

The influence of ionic strength and temperature on the solubilization of lipid bilayers by non-ionic detergents is usually small (7). However, the complex structure of mitochondrial membranes makes conceivable that such variables exert some influence on the solubilizing power of Triton X-100. In order to test this hypothesis, quantitative and qualitative analysis were carried out on the fractions of inner mitochondrial membranes solubilized by Triton X-100 under various con-

ditions of temperature and ionic strength. Small, but significant, changes were observed, that are summarized in the present paper.

Materials and Methods

Rat liver inner mitochondrial membranes were used in all the experiments. The details of membrane preparation and purification have been published previously (6). The purity of membrane preparations was checked by marker enzymes.

The pellet of purified inner mitochondrial membranes was resuspended in a 0.25 M sucrose, 0.02 M HCl-Tris, pH 7.4 buffer to a final concentration of about 4 mg protein/ml. When required, NaCl or $CaCl_2$ were added to a final concentration of 50 or 100 mM. In some cases, EDTA was added to a final concentration of 1 mM. Aliquots of the membrane suspensions were treated with the required amounts of Triton X-100 in order to obtain final detergent concentration of 0.5 % (w/v), and incubated at 4° C or 37° C for 30 min. The membrane suspensions were then centrifuged at 150.000 $\times$ *g* for 60 min at 4° C. The supernatants were considered to contain the solubilized membrane fraction.

Proteins were determined in the presence of Triton X-100 by the method of LOWRY *et al.* (10) as modified by WANG and SMITH (12). Lipids were extracted from the supernatants according to FOLCH *et al.* (3), and lipid phosphorus was determined according to BARTLETT (1). Polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate was performed according to the method of FAIRBANKS *et al.* (2). Cytochromes a, b, c_1 and c were analyzed by differential spectrophotometry of their oxidized and reduced forms (13).

Results and Discussion

In the absence of detergent, the increase in ionic strength due to increasing concentrations of NaCl has no effect on the liberation of proteins (table I). The addition of $CaCl_2$ causes a slight but significant increase in the amounts of solubilized protein. Similar effects of NaCl and $CaCl_2$ can be observed in the presence of 0.5 % Triton X-100 (w/v). The presence of 1 mM EDTA reverses the effects of $CaCl_2$.

For the solubilization of lipid phosphorus, it can be seen that the increase in ionic strength by itself (in the absence of Triton X-100) does not modify the small proportion of lipid P solubilized under those conditions (table I). However, in the presence of 1 mM EDTA, that proportion is considerably increased. The

Table I. *Solubilization of inner mitochondrial membrane protein and lipid phosphorus under various conditions of ionic strength, in the presence and absence of 0.5 % Triton X-100, at 20° C.*

Figures correspond to mean values ± S.E.M. of the number of experiments indicated in parenthesis. * $p < 0.01$; ** $p < 0.05$.

Additions	% solubilized protein		% solubilized lipid P	
	Control	+ Triton X-100	Control	+ Triton X-100
None	13.1±0.46 (46)	42.6±1.18 (46)	4.4±0.92 (13)	58.7±5.71 (9)
50 mM NaCl	13.1±1.14 (11)	42.3±2.88 (8)	5.8±1.91 (9)	72.6±5.14 (5)
100 mM NaCl	13.9±1.35 (11)	43.8±2.46 (8)	5.0±1.20 (8)	68.0±2.51 (4)
50 mM $CaCl_2$	14.5±0.98 (10)	50.2±3.44 (7)**	4.2±0.81 (9)	67.9±3.39 (5)
100 mM $CaCl_2$	15.2±0.63 (9)*	50.7±4.57 (6)**	5.3±1.35 (8)	61.7±1.59 (4)
1 mM EDTA	12.5±0.56 (11)	46.1±2.66 (10)	12.5±1.94 (4)*	74.9±5.07 (4)

interpretation of this phenomenon remains nuclear, and deserves further investigation. The proportion of lipid P solubilized by 0.5 % Triton X-100 is slightly increased in the presence of NaCl. Interestingly, $CaCl_2$ has the opposite effect, which is in turn reversed by 1 mM EDTA, the latter increasing again the proportion of solubilized lipid P. The effects of Ca^{++} and EDTA might be related to the lateral seggregation of acid phospholipids induced in the plane of the lipid bilayer by that cation (4). Such seggregation would compensate the effect of increasing ionic strength, i.e. increase in lipid P solubilization, observed with NaCl. The latter might act through changes in the disposition of peripheral proteins.

Temperature changes do not affect the small proportion of proteins or phospholipids that come into solution in the experiments performed in the absence of Triton X-100 (table II). It is interesting to observe that increasing temperatures tend to decrease, although not significantly, the proportion of protein solubilized by the detergent. This could be attributed to the temperature-dependent increase in bond energy of the hydrophobic bonds (11) that are loosened by the detergent. The situation is more complex with regard to the influence of temperature on the solubilization of lipid phosphorus by the detergent, since the proportion solubilized at 20° C is smaller that at either 4° C or 37° C.

Polyacrylamide gel electrophoresis was performed on the solubilized fractions of inner mitochondrial membranes treated under different conditions of temperature and ionic strength. The supernatants corresponding to control and detergent-treated membranes were analyzed. No differences could be found in any of the electrophoretic patterns attributable to temperature or ionic strength. However, this does not preclude the more or less specific solubilization of some peptides under those conditions, since the complexity of the membrane polypeptide composition exceeds by far the resolving capacity of the technique.

In order to get some information about possible qualitative differences in the membrane components solubilized under various conditions of ionic strength, individual cytochromes were determined in such experiments (table III). No large effects were seen for cytochromes *a* or *b* but, as expected from their extrinsic character, 50 or 100 mM NaCl markedly increased the solubilization of cytochromes *c* and c_1 by 0.5 % Triton X-100. This effect seems to be largely, though not entirely, due to the ability of salts to detach these cytochromes from the membrane in the absence of detergent.

The effect of EDTA tends in general to decrease the proportion of cytochromes solubilized by the detergent, while, by itself, it has little or no effect. The decrease in detergent-solubilized material is more evident in the cases of cytochromes *b* and c_1. This effect suggests some cal-

Table II. *Solubilization of inner mitochondrial membrane protein and lipid phosphorus by 0.5 % Triton X-100, at various temperatures.*
Figures correspond to mean values ± S.E.M. of the number of experiments indicated in parenthesis.

Incubation temperature	% solubilized protein		% solubilized lipid P	
	Control	+ Triton X-100	Control	+ Triton X-100
4°	11.6±0.65 (10)	44.7±3.69 (9)	4.4±1.13 (7)	68.4±5.74 (5)
20°	13.1±0.46 (46)	42.6±1.18 (46)	4.4±0.92 (13)	58.7±5.70 (15)
37°	13.6±0.88 (10)	39.4±4.97 (9)	5.2±1.22 (9)	67.4±2.58 (5)

Table III. *Solubilization of various cytochromes from the inner mitochondrial membranes by 0.5 % Triton X-100 under various conditions of ionic strength.*
Figures correspond to mean values ± S.E.M. of five independent experiments. * $p < 0.01$; ** $p < 0.005$.

Additions	% solubilization							
	Cyt. a		Cyt. b		Cyt. c_1		Cyt. c	
	Control	+ 0.5 % Triton X-100	Control	+ 0.5 % Triton X-100	Control	+ 0.5 % Triton X-100	Control	+ 0.5 % Triton X-100
None	4.0±2.10	18.8±6.21	4.1±1.65	46.5± 9.16	4.5±2.90	46.4± 9.31	8.4± 5.00	31.6± 4.52
50 mM NaCl	4.0±2.92	17.2±7.60	4.2±2.00	55.8± 5.61	17.6±4.36*	50.3±12.20	19.7± 5.61	60.0±11.12*
100 mM NaCl	7.8±6.70	18.2±5.53	9.0±5.52	67.8±11.60	30.6±9.70*	62.9± 9.33	45.5±18.20	89.0± 3.17**
1 mM EDTA	8.7±6.41	14.8±7.81	13.5±6.71	22.5± 8.20	11.0±9.10	14.8± 8.11*	4.2± 3.60	24.5±14.00

cium-dependent conformational change in relation to the solubilization of those cytochromes, or cytochrome chromophores. A direct study of the influence of Ca^{++} on cytochrome solubilization could not be carried out because of the formation of insoluble complexes with the bile salts that are used in the cytochrome assays. A relatively high variability is observed in the cytochrome experiments. This is due to the small concentration of cytochromes in the supernatants containing the solubilized fraction, and to the high light-scattering effect of the resuspended non-solubilized material.

According to the data presented here, ionic strength and temperature affect in some way the solubilization of cell membranes by non-ionic detergents. This could be due, among other circumstances, to: *a*) perturbation of the non-covalent bonds that hold together the different membrane components; *b*) removal of extrinsic proteins, thus allowing the detergent to act on otherwise «hidden» areas, or *c*) conformational changes of the membranes, or of membrane components, also modifying the domains accessible to the surfactant.

Acknowledgements

The author is grateful to Dr. F. M. Goñi for his advice and criticism throughout this work.

This research was supported in part with funds from the Spanish «Comisión Asesora para la Investigación Científica y Técnica.

Resumen

Membranas internas mitocondriales de hígado de rata se someten a la acción solubilizante del detergente no iónico Triton X-100 en distintas condiciones de fuerza iónica y temperatura. El aumento de fuerza iónica tiene escasa influencia en la cantidad de proteína y lípidos de membrana solubilizados. El cloruro cálcico aumenta la proporción de proteína solubilizada, y lo contrario ocurre en presencia de EDTA 1 mM. El aumento de temperatura tiende a disminuir la proporción de proteína solubilizada por el detergente. La electroforesis en gel de poliacrilamida en presencia de SDS no detecta diferencias entre los polipéptidos solubilizados en unas u otras condiciones. Sin embargo, se observan diferencias al estudiar la solubilización de los distintos citocromos. Los datos se interpretan como resultado de las variaciones en la arquitectura de la membrana, inducidas por las distintas condiciones del medio de incubación.

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