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Glycyl betaine is effective in slowing down the irreversible denaturation of a detergent-solubilized membrane protein, sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA1a)

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ABSTRACT

Many membrane proteins become labile when they are solubilized by detergent. Here we show that the presence of high concentrations of glycyl betaine stabilizes one of these proteins, the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA1a), solubilized with nonionic detergents like n-dodecyl β-D-maltopyranoside (DDM) or octaethylene glycol monododecyl ether ($C_{12}E_8$) which are commonly used for its purification or crystallization. Betaine at high concentrations might become useful as a stabilizing agent for detergent-solubilized membrane proteins, for instance during purification procedures or during the long periods of time required for crystallogenesis.

Introduction

Like many other membrane proteins, SERCA1a, the ATP-dependent Ca^{2+} pump of sarcoplasmic/endoplasmic reticulum in skeletal muscle cells [1,2], becomes fairly unstable after solubilization by detergent (*e.g.* [3] and references herein). Such irreversible inactivation of solubilized SERCA1a has already been shown to be slowed down by including glycerol in the solubilization buffer (see references in [4]). Independently, various molecules, sometimes called "cosmotropic" osmolytes (*e.g.* polyols, sugars, certain amino acids or other molecules), have been reported at high concentrations to protect the native structure of water-soluble proteins, possibly by favouring compact states for these proteins because of exclusion of these osmolytes from the water-protein interface [5]. One of these molecules, glycyl betaine (or betaine, for simplicity), an effective enhancer of the tolerance of plants (including the sugar beet *Beta vulgaris* L.) against abiotic stress [6], was reported to protect the Photosystem II complex from dissociation of its extrinsic subunits [7,8], and to protect two different Ca^{2+} pumps from perturbation by urea [9,10]. Here we describe the previously unnoticed protecting effect exerted by betaine on detergent-solubilized SERCA1a. The use of this stabilizing agent for protection of detergent-solubilized membrane proteins in general (and SERCA1a in particular) might prove useful during isolation and purification procedures in which glycerol viscosity would otherwise severely slow down steps like chromatography or ultrafiltration. By making it possible to perform crystallogenesis attempts under new conditions, its use might also lead to new crystalline forms for these detergent-solubilized membrane proteins.

Materials and methods

Sarcoplasmic reticulum (SR) membrane fragments were prepared as previously described [11]. The nonionic detergents $C_{12}E_8$ (octaethylene glycol monododecyl ether) and DDM (*n*-dodecyl β-D-maltopyranoside) were from Nikko Chemicals Ltd. (Japan) and Anatrace Inc. (USA), respectively. DOPC (dioleoyl phosphatidylcholine) was from Avanti Polar Lipids (USA). Glycyl betaine (thereafter referred to as "betaine", for simplicity) was from Fluka. The betaine stock solution was prepared by adding 12 g of solid betaine to 15 g of buffer A (100 mM KCl, 50 mM Tes–Tris and 1 mM Mg^{2+}, at pH 7.5 and 20 °C), resulting in a final volume of about 25 ml and therefore a final betaine concentration of about

Abbreviations: SERCA1a, sarco-endoplasmic reticulum Ca^{2+}-ATPase, isoform 1a; SR, sarcoplasmic reticulum; ATPase, adenosine triphosphatase; EGTA, [ethylenebis-(oxyethylenenitrilo)]tetraacetic acid; Tes, *N*-tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid; Tris, tris(hydroxymethyl)aminomethane; $C_{12}E_8$, octaethylene glycol mono-*n*-dodecyl ether; DDM, *n*-dodecyl β-D-maltopyranoside; DOPC, 1,2-dioleoyl phosphatidylcholine; cmc, critical micellar concentration.

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4 M (together with concentrations of KCl, Tes and Mg^{2+} lower by about 40% than in the initial buffer A). The residual ATPase activity of SERCA1a after incubation of SR membrane fragments in the presence or in the absence of detergent was deduced from the Ca^{2+}-dependent fraction of the rate of NADH oxidation measured in a coupled enzyme assay, with the assay medium consisting of buffer A supplemented with 0.1 mM Ca^{2+}, 0.05 mM EGTA, 5 mM MgATP, 1 mg/ml $C_{12}E_8$, 0.1 mg/ml pyruvate kinase, 0.1 mg/ml lactate dehydrogenase, 1 mM phosphoenol pyruvate and an initial concentration of 0.2 to 0.3 mM NADH [3].

Results and discussion

We found that the long-known time-dependent irreversible inactivation observed upon incubation of detergent-solubilized SERCA1a for a few minutes in the absence of Ca^{2+} ([3,4] and references herein) was slowed down to a significant extent by including betaine in the solubilizing incubation medium (Fig. 1). This betaine-dependent stabilization of the Ca^{2+}-free solubilized SERCA1a occurred for both $C_{12}E_8$- and DDM-solubilized SERCA1a (Panels A and B in Fig. 1). It also occurred after solubilization at a reduced detergent/protein concentration (Panel C), *i.e.* when reduced delipidation already protects SERCA1a from irreversible denaturation to a significant extent ([3] and references herein). Betaine concentrations enhanced stability up to 1 M, while higher betaine concentrations were less effective (Panel D). Specific effects of betaine on the *function* of SERCA1a inserted in its natural membrane environment have already been described [12].

We also tested the stabilizing effect of 1 M betaine in the presence of Ca^{2+}, *i.e.* under conditions where time-dependent inactivation of the solubilized SERCA1a is much slower [3,4]. Betaine was again helpful, over very long periods, and in fact even more helpful on a relative basis: over a few days at either 20 or 6 °C, betaine was as potent as 20% v/v glycerol (about 2.8 M) in stabilizing SERCA1a solubilized with either $C_{12}E_8$ or DDM (Panels A–C in Fig. 2), irrespective of the temperature (note that time-dependent irreversible inhibition of $C_{12}E_8$-solubilized SERCA1a is slower at 20 °C than at 6 °C, as previously mentioned to be the case for DDM-solubilized SERCA1a [13]). The protecting effect afforded by betaine was similar to or even higher than the one afforded by including lipid together with detergent (see Panel D). In separate controls, we checked by fluorescence measurements with anilinonaphthalene sulfonate as well as by elastic light scattering measurements that betaine did not significantly alter either the critical micellar concentration of $C_{12}E_8$ and DDM or the ability of these detergents to solubilize SR membranes (data not shown).

Protection of the solubilized SERCA1a by betaine, although not 100% in the absence of Ca^{2+} (Fig. 1), was quite impressive in the presence of Ca^{2+}, even in the absence of lipid (Fig. 2). Betaine at

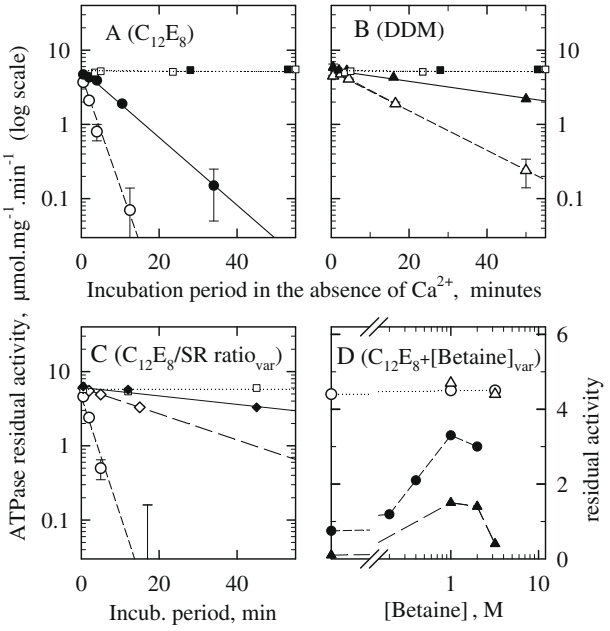

Fig. 1. Protection conferred by glycyl betaine against irreversible inactivation of detergent-solubilized Ca^{2+}-free ATPase. SR membranes (1 mg/ml SR protein) were incubated in a medium containing 100 mM KCl, 50 mM Tes–Tris, 1 mM Mg^{2+} at pH 7.5 and 20 °C (buffer A) in the quasi-absence of free Ca^{2+} (0.1 mM total Ca^{2+} and 1 mM EGTA, Ca^{2+}-free ~5 nM), in the absence or presence of detergent and in the absence or presence of glycyl betaine. The detergent was either $C_{12}E_8$ or DDM, at a concentration (if present) of 5 mg/ml, except for Panel C for which 1.5 mg/ml was also used. The concentration of glycyl betaine (if present) was 1 M, except for Panel D for which various concentrations were used, as indicated on the abscissa. After various incubations periods, aliquots were diluted 250-fold into a $C_{12}E_8$-containing ATPase assay medium. (A) Circles, incubation in the presence of 5 mg/ml $C_{12}E_8$, in the absence (open symbols) or presence (closed symbols) of 1 M betaine; controls were performed in the absence of detergent (squares). (B) Triangles, incubation in the presence of 5 mg/ml DDM, in the absence (open symbols) or in the presence (closed symbols) of 1 M betaine; squares correspond to the previously mentioned controls in the absence of detergent. (C) Diamonds, incubation was performed in the presence of 1.5 mg/ml $C_{12}E_8$, in the absence or presence of 1 M betaine (open and closed symbols, respectively); separate incubations in the absence of betaine and absence or presence of 5 mg/ml $C_{12}E_8$ were also performed for control (open squares and circles, respectively). (D) Closed symbols, incubation was performed in the presence of 5 mg/ml $C_{12}E_8$ and various concentrations of betaine; the residual ATPase activity was measured after either 5 min (circles) or 15 min (triangles). Controls incubated in the absence of detergent were also included (open symbols).

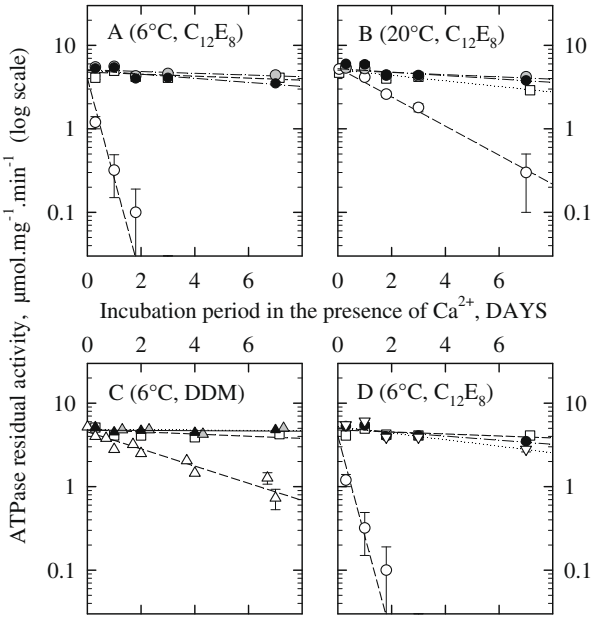

Fig. 2. Protection conferred by glycyl betaine (compared with glycerol or lipids) against irreversible inactivation of detergent-solubilized Ca^{2+}-saturated ATPase. SR membranes (1 mg/ml SR protein) were incubated in a medium containing 80 mM KCl, 40 mM Tes–Tris, 0.8 mM Mg^{2+} and 1 mM Ca^{2+}, in the absence (squares) or in the presence of 5 mg/ml detergent, either $C_{12}E_8$ (circles, Panels A, B and D) or DDM (triangles, Panel C), and in the absence (open symbols) or presence of either glycyl betaine at 1 M (closed symbols) or glycerol at 20% v/v (gray symbols). Temperature was either 6 °C (Panels A, C and D) or 20 °C (Panel B), with pH set at 7.5 in both cases. Panel D reproduces some of the data points from Panel A and compares them with the results obtained in an experiment (inverted triangles) where the incubation medium contained dioleoyl phosphatidylcholine (DOPC) in addition to $C_{12}E_8$ (2 mg/ml and 5 mg/ml, respectively), in the absence of betaine. After various periods, aliquots were diluted 250-fold into a $C_{12}E_8$-containing ATPase assay medium for residual ATPase activity measurement, as for Fig. 1 experiments. Panels A and D, incubation with $C_{12}E_8$ at 6 °C; Panel B, incubation with $C_{12}E_8$ at 20 °C; Panel C, incubation with DDM at 6 °C; for this Panel, [Ca^{2+}] during incubation was only 0.8 mM.

high concentrations might indeed become useful as a stabilizing agent for detergent-solubilized SERCA1a, and probably for other membrane proteins, too, as suggested also by the above-quoted results with Photosystem II [7–8]. Betaine, instead of glycerol or together with a reduced concentration of glycerol, could for instance prove useful for the purification of detergent-solubilized membrane proteins by procedures in which the intrinsic viscosity of glycerol would slow down and possibly prevent operation (*e.g.* for column equilibration and elution with glycerol-containing media, or for ultrafiltration). Betaine, which is already proposed in commercial crystallization screens as one out of many additives to be used at low (millimolar) concentrations, could also be used at higher concentrations to stabilize detergent-solubilized proteins during the lengthy incubation periods required for their crystallization, to hopefully lead to the discovery of new crystalline forms, or perhaps to higher resolution crystals.

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