

## Inactivation of the Sarcoplasmic Reticulum Calcium-Transport-ATPase by Lasolocid in Combination with Triton X-100

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The calcium-transport-ATPase of the sarcoplasmic reticulum membranes is irreversibly inactivated by the combined action of Lasolocid and Triton X-100 at concentrations which separately do not interfere with the enzyme's activity. In the presence of Lasolocid the enzyme is most susceptible to inactivation when the Triton X-100 concentration just exceeds its critical micellar concentration,  $\sim 0.2 \text{ mg} \cdot \text{ml}^{-1}$ . Lasolocid becomes effective at a concentration of  $10 \mu\text{M}$  and produces rapid inactivation at  $100 \mu\text{M}$ . The enzyme is more rapidly inactivated in the active than in the inactive state.

### Introduction

Various antibiotics isolated from different molds (e.g. A 23187 or X 537A named Lasolocid) specifically increase the calcium permeability of biological membranes and artificial bilayers [1, 2]. The assumption that the ionophore acts as a mobile carrier has been proven for synthetic bilayers [3, 4]. Furthermore Pringle and Hidalgo demonstrated that A 23187 neither affects the rotational motion of the calcium ATPase from sarcoplasmic reticulum, nor the mobility of the lipid acyl chains of the membrane phospholipids [5]. In contrast to the sarcoplasmic reticulum an interaction of A 23187 with intrinsic membrane proteins has been proposed in lymphocyte membranes [6]. Therefore it appears that the interaction of an ionophore with membrane proteins might specifically depend on the nature of the lipids and the proteins in the respective membrane. In fact a direct interaction of Lasolocid with the calcium transport ATPase can be demonstrated in Triton X-100 perturbed sarcoplasmic reticulum membranes.

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## Materials and Methods

Sarcoplasmic reticulum vesicles were prepared according to the procedure described by Hasselbach and Makinose [7] as modified by de Meis and Hasselbach [8]. Protein concentration was determined by the biuret method calibrated by Kjeldahl standards.

### Assays

$0.05 \text{ mg}$  sarcoplasmic reticulum vesicles per ml were suspended in solutions containing  $40 \text{ mM}$  KCl,  $50 \text{ mM}$  imidazole,  $\text{pH } 7$ ,  $20 \text{ mM}$   $\text{MgCl}_2$ ,  $0.1 \text{ mM}$   $\text{CaCl}_2$ ,  $0.1 \text{ mM}$  dithioerythritol and  $0.4 \text{ mM}$  dinitrophenylphosphate. The dinitrophenylphosphatase activity was measured spectrophotometrically at  $412 \text{ nm}$  and  $25^\circ\text{C}$ , for  $2\text{--}3 \text{ min}$ . Subsequently, Triton X-100 up to  $1 \text{ mg} \cdot \text{ml}^{-1}$  and/or Lasolocid ( $10\text{--}100 \mu\text{M}$ ) were added to the assay and the enzyme's activity was monitored for several minutes [9]. In another experimental set up dinitrophenylphosphate hydrolysis was initiated after the vesicles were incubated for various times with Triton and/or Lasolocid.

Lasolocid (X 537A) was a generous gift of Hoffmann-La Roche Ltd., Basel (Switzerland), Triton X-100 was obtained from Serva, Heidelberg (FRG), dinitrophenylphosphate was synthesized according to Ramirez and Marecek [10] and used as its lutidinium salt.

## Results and Discussion

As shown in Fig. 1A the calcium-dependent hydrolysis of dinitrophenyl phosphate of closed sarcoplasmic reticulum vesicles increases  $4\text{--}5$  fold when  $1 \text{ mg} \cdot \text{ml}^{-1}$  Triton X-100 or  $100 \mu\text{M}$  Lasolocid are added to the assay medium. At these concentrations the agents produce the same maximal enhancement of enzymatic activity. It is well established that the activating effect of both agents is causally linked to the decline of the concentration of internally stored calcium, which effectively depresses substrate hydrolysis (cf. 11). The enzyme's activity displays a complete different time course when both agents were present simultaneously (Fig. 1B). The enzymatic activity continuously declines and approaches



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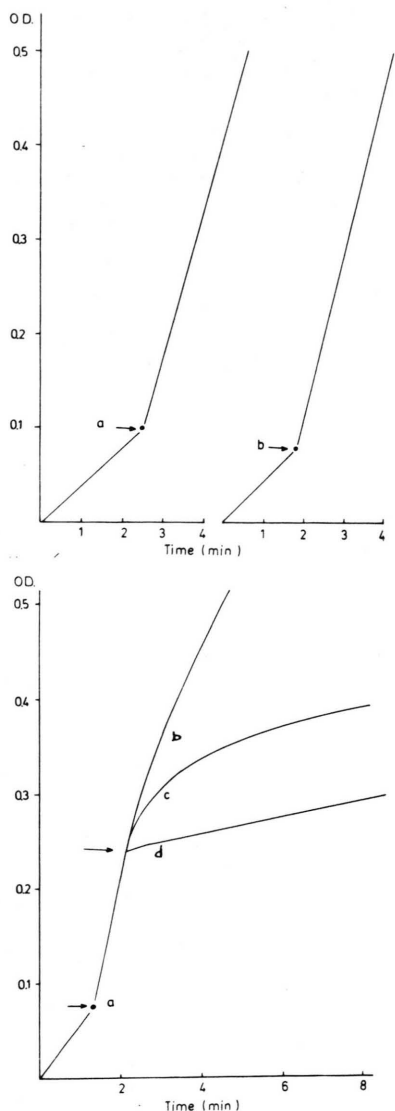


Fig. 1. A. The increment of dinitrophenolphosphate hydrolysis of sarcoplasmic reticulum vesicles produced by Triton X-100 or Lasolocid. The assay media is described in "Materials and Methods": a) Addition of Triton X-100, final concentration  $1 \text{ mg} \cdot \text{ml}^{-1}$ . The rate of hydrolysis increased from  $0.13$  to  $0.55 \text{ } \mu\text{mol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ . b) Addition of Lasolocid, final concentration  $0.1 \text{ mM}$ , the rate of hydrolysis increased from  $0.14$  to  $0.6 \text{ } \mu\text{mol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ . B. Abolition of dinitrophenolphosphate hydrolysis of sarcoplasmic reticulum vesicles by Triton X-100 in combination with Lasolocid. a) On addition of Triton X-100,  $\rightarrow$  final concentration  $0.2 \text{ mg} \cdot \text{ml}^{-1}$  the hydrolysis rises from  $0.18$  to  $0.7 \text{ } \mu\text{mol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ . On addition of Lasolocid  $\rightarrow$  b)  $20 \text{ } \mu\text{M}$ , c)  $40 \text{ } \mu\text{M}$ , d)  $100 \text{ } \mu\text{M}$  the enzymatic activity starts to decline progressively. The same time course of inactivation is observed if Lasolocid is added prior to Triton X-100.

very low residual values. Time course and extent of this spontaneous inactivation are independent on the order of addition of the agents but depend on the concentrations of both Lasolocid and Triton X-100. The minimal effective concentration of Lasolocid is  $10 \text{ } \mu\text{M}$  and addition of  $100 \text{ } \mu\text{M}$  abolishes the enzymatic activity in less than  $1 \text{ min}$ . Triton X-100 affects the enzyme in a more complex manner. Concentrations lower than  $0.1 \text{ mg} \cdot \text{ml}^{-1}$  produces only little inactivation in the presence of Lasolocid. A rapid inactivation is already achieved at  $0.2 \text{ mg} \cdot \text{ml}^{-1}$ . This is just above the critical micellar concentration ( $0.15 \text{ mg} \cdot \text{ml}^{-1}$ , [12]). At this concentration the ATPase protein which is present at only  $0.05 \text{ mg} \cdot \text{ml}^{-1}$  is largely stripped of its native lipids, whereby mixed lipid Triton X-100 micelles are formed. The amount of non-lipid bound Triton X-100 is presumably sufficient to just cover the hydrophobic surface area of the protein [13, 14]. In this state where the native lipids are removed and marginally replaced by Triton X-100 the enzyme becomes Lasolocid sensitive. When the Triton X-100 is further increased inactivation is progressively retarded. In this case, Lasolocid might preferably combine with Triton X-100 micelles present in excess.

The described decay of enzymatic activity occurs more rapidly in the active than in the inactive enzyme. As shown in Fig. 1B, curve c, at  $40 \text{ } \mu\text{M}$  Lasolocid and  $0.2 \text{ mg} \cdot \text{ml}^{-1}$  Triton X-100 enzymatic activity is completely abolished during a  $6 \text{ min}$  period of dinitrophenylphosphate splitting. On the other hand, if the enzyme is incubated  $30 \text{ min}$  in  $40 \text{ } \mu\text{M}$  Lasolocid and  $0.3\text{--}0.5 \text{ mg} \cdot \text{ml}^{-1}$  Triton X-100 without any substrate, enzymatic activity is still  $40\%$  of the maximal activity shown in Fig. 1, if measured immediately after the addition of dinitrophenylphosphate. One might assume that the enzymatic activity changes the pattern of hydrophobic interaction between protein and Triton X-100, making the protein accessible for Lasolocid.

Finally it should be noted, that (i) the same results are obtained if instead of dinitrophenyl phosphate, ATP was used as substrate; (ii) in contrast to Lasolocid the ionophore A 23187 is not effective (ii) Triton X-100 can not be replaced by lysolecithins or lauryloctylethylene glycol ( $\text{C}_{12}\text{E}_8$ ).

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