*Biochimica et Biophysica Acta*, 483 (1977) 483—486


BBA Report

BBA 61321

# RESTORATION OF DETERGENT-INACTIVATED ADENOSINE TRIPHOSPHATASE ACTIVITY OF HUMAN PROSTATIC FLUID WITH CONCANAVALIN A

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(Received March 8th, 1977)

## Summary

A stimulation by concanavalin A of $Mg^{2+}$- and $Ca^{2+}$-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) of human prostatic fluid has been observed after the enzyme system had been inactivated by a detergent such as 0.05% deoxycholate. The concanavalin A effect was specific since the positive effect was abolished in the presence of α-methyl-D-mannoside. Furthermore, the positive effect of concanavalin A was obtained with a low lectin concentration, equal to the concentration reported for optimal stimulation of other membrane enzymes.

The presence of a $Mg^{2+}$- and $Ca^{2+}$-dependent adenosine triphosphatase (ATPase) (ATP phosphohydrolase, EC 3.6.1.3) in prostatic fluid was demonstrated in a recent work [1]. This ATPase was bound to a pellet (pellet II), obtained upon high-speed centrifugation after the spermatozoa and cell debris (pellet I) had been removed in a preceeding preparatory step [1]. The pellet II material was studied in the electron microscope and found to contain secretory granules and vesicles to which the ATPase activity was most probably associated [1]. This ATPase was characterized in several respects and among other things found to be inhibited by detergents such as deoxycholate, Triton X-100 and dodecyl sulfate [1].

We have examined the detergent inactivation further and have found to our surprise that the detergent-inactivated pellet II material can be reactivated with respect to its ATPase activity by the addition of small amounts of concanavalin A (Con A*) to the incubation system.

*Pharmacia Fine Chemicals, Uppsala, Sweden. Freeze-dried. Prepared by chromatography on Sephadex. Carbohydrate < 0.1%.

TABLE I

$Mg^{2+}$- AND $Ca^{2+}$-DEPENDENT ADENOSINE TRIPHOSPHATASE ACTIVITIES OF HUMAN PROSTATIC FLUID UNDER VARIOUS CONDITIONS

The pellet II material was prepared from the prostatic fluid as has been referred to in the text. An ordinary incubation medium (experiment 1) contained the following: 0.7 mM ATP, 2 mM $MgSO_4$ ($Mg^{2+}$-ATPase) or 2 mM $CaCl_2$ ($Ca^{2+}$-ATPase), 1 mM glutathione (reduced form), pellet II material containing 25 μg protein (which corresponds to 50 μl of prostatic fluid) and 50 mM triethanolamine · HCl buffer, pH 7.6. The same medium was also used for experiment 2, but 80 mM α-methyl-D-mannoside (Me-Man) was included (except D). Total incubation volume was 3.0 ml and incubation was proceeded for 10 min at 37°C. 1A and 2A: Treatment of pellet II material in the ordinary incubation medium lacking ATP with 0.05% deoxycholate, sodium salt (DOC) for 10 min at 20°C before incubation at 37°C which was started with addition of ATP. 1B and 2B: Treatment of pellet II material in the ordinary incubation medium lacking ATP with concanavalin A (Con A, 16 μg/ml) for 45 min at 37°C. Incubation was started with the addition of ATP. 1C and 2C represent first a 10 min treatment at 20°C with 0.05% deoxycholate followed by 45 min at 37°C with Con A (16 μg/ml) in the incubation medium lacking ATP. Incubation was started with the addition of ATP. 2D: Similar to 2C, but without α-methyl-D-mannoside.

| Experiment | Addition | Activity (nmol orthophosphate liberated · 0.1 $ml^{-1}$ · $min^{-1}$) | |
|---|---|---|---|
| | | $Mg^{2+}$-ATPase | $Ca^{2+}$-ATPase |
| 1 | None | 187 | 194 |
| A | 0.05% DOC | 42 | 48 |
| B | Con A | 187 | 187 |
| C | 0.05% DOC + Con A | 187 | 154 |
| 2 | Me-Man | 207 | 211 |
| A | Me-Man + 0.05% DOC | 45 | 50 |
| B | Me-Man + Con A | 207 | 210 |
| C | Me-Man + 0.05% DOC + Con A | 42 | 43 |
| D | 0.05% DOC + Con A | 167 | 167 |

The prostatic fluid was obtained and the pellet II material was prepared as has been described previously [1]. $Mg^{2+}$-dependent and $Ca^{2+}$-dependent ATPase activity measurements were also in accordance with the previous work [1]. Table I demonstrates the typical 75% inhibition of the $Ca^{2+}$-dependent ATPase on pre-incubation for 10 min at 20°C of the pellet II material suspended in triethanolamine buffer, pH 7.6, also containing 0.05% deoxycholate. If Con A is added to the medium together with glutathione and $Mg^{2+}$ or $Ca^{2+}$ but without ATP for the incubation for 45 min at 37°C and ATP is thereafter added at the end of the 45 min period, the inactivated $Ca^{2+}$-dependent ATPase is again reactivated by 83%. It is also seen in Table I that Con A itself in the incubation medium in the absence of deoxycholate has a negligible inhibitory effect on the $Ca^{2+}$-dependent ATPase consistent with our previous finding [1].

The same pattern is also seen for the $Mg^{2+}$-dependent ATPase. The corresponding inactivation with 0.05% deoxycholate is 78% and the reactivating ability of Con A is in this case 100%. If the sequence of additions is reversed prior to incubation with ATP, i.e. Con A is added before deoxycholate, there is no inhibitory action at all by deoxycholate. Con A binds to saccharides that contain α-D-mannopyranosyl or α-D-glucopyranosyl residues [2, 3] and to a variety of cell surfaces [4–9]. It has also been claimed that this lectin can interact with specific glycolipids in bilayer membranes [10]. Such an interaction is not only confined to the lipid hydrophilic surface but involves also modifications inside the hydrophobic part of the bilayer structure [10]. Recently the binding of Con A to other membrane systems was also demonstrated, such as to the membranes of the Golgi apparatus [11], and to the

nuclear membranes of rat liver cells [12]. The binding of Con A to the carbohydrate moiety of the membrane can be prevented by α-methyl-D-mannoside. To investigate the specificity of the Con A effects in our system, we also performed experiments in the presence of 80 mM α-methyl-D-mannoside*. The experiments were identical with those described above with the difference that the aforementioned glycoside was present from the beginning. It is seen in Table I that neither the $Ca^{2+}$-dependent nor the $Mg^{2+}$-dependent ATPase is influenced by the glycoside. Consequently the same ATPase activities are displayed in the presence of Con A together with the glycoside. Also, the pretreatment of the pellet II material with 0.05% deoxycholate in the presence of 80 mM of α-methyl-D-mannoside results in a 79% inactivation of the $Ca^{2+}$-dependent ATPase and 76% inactivation of the $Mg^{2+}$-dependent ATPase. These figures are congruous with those obtained with deoxycholate in the absence of the glycoside. However, if Con A is added following the deoxycholate treatment as before but in the presence of the glycoside, no reactivation at all is obtained of either the $Ca^{2+}$-dependent or the $Mg^{2+}$-dependent ATPase. The reactivating effect of Con A after treatment with deoxycholate is possible only if the glycoside is not present in the incubation medium. Thus, we have demonstrated in the present work the ability of Con A to reactivate a deoxycholate-inactivated $Mg^{2+}$- and $Ca^{2+}$-dependent ATPase system from human prostatic fluid. The effect is presumably exerted via an interaction with a carbohydrate moiety, since reactivation can be prevented by α-methyl-D-mannoside. This detergent inactivation followed by reactivation by Con A is not unique for deoxycholate, since the same type of result has also been achieved with Triton X-100 and dodecyl sulfate.

The interaction of Con A with membranes appears to involve very specific and complex interactions, which can ultimately lead to a variety of biological effects [3]. Most of the studies have been performed on lymphocytes. Acyl CoA:lysolecithin acyltransferase (EC 2.3.1.23) is a membrane-bound enzyme that is activated directly after binding of Con A [13, 14] resulting in increased turnover of the fatty acids of membrane phospholipids. Furthermore, this enzyme exhibits a high affinity for polyunsaturated fatty acids and this mechanism therefore leads to a higher content of polyunsaturated membrane phospholipids [14]. An enhanced turnover of phosphatidylinositol has also been reported to occur as a consequence of Con A stimulation [15, 16]. 5′-Nucleotidase has been claimed to be inhibited by Con A in different animal cells while the $Mg^{2+}$-dependent ATPase has been stimulated [17—19]. All these enzymatic effects by Con A seem to be rather momentaneous and do not involve the genome and protein synthesis steps. Also, the reactivating effect studied by us is rather momentaneous. There is no simple explanation for this reactivation. The effect is specific, since α-methyl-D-mannoside nullified it. Furthermore, the concentration of Con A was low and comparable with the concentration used for achieving maximal stimulation of the acyl CoA:lysolecithin acyltransferase [20].

At least two mechanisms for the mediation by Con A of the increased expression of ATPase in the membranous material after inactivation with deoxy-

*From Sigma Chemical Company, St. Louis, Mo., U.S.A., grade III, 99%.

cholate are compatible with the present data. One would envision the binding of Con A to a surface carbohydrate inhibitor of the ATPase system. This inhibitor is normally not directly associated with the membrane-bound ATPase system due to the presence of neighbouring phospholipids. This kind of inhibition is only possible after removal of the phospholipids by a detergent and the inhibitory action can then be abolished by Con A binding to the carbohydrate inhibitor.

The other mechanism postulates a Con A binding to the enzyme system leading to stimulation. Con A would in such a case be a positive allosteric effector. This would be in agreement with results showing that membrane-bound enzymes such as ATPases display cooperativity towards various allosteric effectors [21—25]. It is of special interest to note that the degree of such a cooperativity of the enzyme system (as expressed by the Hill coefficient) is a function of the ratio of unsaturated/saturated fatty acids of membrane phospholipids. In this context it is worthy of note that Con A has also been reported to exert a hormone-like action at the cell surface, thus mimicking hormonal stimulation of metabolic events in certain types of cells [26].

## Acknowledgements

This investigation was financially supported by a grant from the Södermanland Country Council, Sweden. We thank Dr A. Gottfries for kindly providing us with the prostatic fluid material.

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