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PHOSPHOLIPID INTERACTIONS WITH A XYLOSYL-TRANSFERASE FROM AORTIC WALL

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SUMMARY : In aortic wall a xylosyltransferase catalyzes the transfer of [^{14}C]-xylose from UDP-[^{14}C]-xylose onto endogenous or exogenous acceptor (poly-L-serine). The purification of this enzyme is carried out by hydrophobic chromatography onto an octyl-agarose column which conducts to the separation of the endogenous acceptor from the enzyme. The xylosyltransferase is markedly inhibited by phospholipids such as phosphatidic acid, phosphatidyl-L-serine and lysophosphatidylcholine. Our investigations upon synthetic phospholipids or analogs allow us to show that this change in enzymatic activity is neither stereospecific nor dependent on the steric hindrance of phospholipidic compounds but is related to their polar headgroups, particularly to acidic groups of phosphatidylserine or phosphatidic acid. Detergents ionic or not interfere also as inhibitors. Exogenous phospholipids certainly induce some modifications in the enzymatic environment which could either lead to an enzymatic conformation change by preventing its aggregation or could interfere directly on the xylosyltranferase activity.

INTRODUCTION

Previous investigations have shown the occurence of glycosyltransferases in the intima-media cells of aortic wall. Some of these enzymes have been isolated from cell-sap as xylosyltransferase : E.C. 2.4.2.26 [1] and galactosyltransferase : E.C. 2.4.1.38 [2] involved in glycosamino-glycurono-glycans and in glycoprotein biosynthesis. Our studies for the xylosyl-transferase were carried out onto endogenous acceptor [1] and exogenous acceptor (poly-L-serine [3]) after its purification by isoelectric focusing in ampholine column. This technic conducted to a loss of enzymatic activity, so we report in this paper a purification by hydrophobic chromatography. As lipidic compounds interacted with this enzyme [4], currently we report further attempts in order to deepen our knowledge about the relationship between the molecular structure of phospholipids and the related effect observed on the glycosylation processes.

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MATERIALS AND METHODS

- Chemicals

Phospholipids extracted from natural substances were purchased from Sigma, while synthetic phospholipids or analogs were brought about Medmark (Munich), P.L. Biochemicals Inc (Milwaukee), Serdary Research laboratories (London, Ontario). Hydrophobic chromatography kits, octyl-agarose and poly-L-serine were purchased from Miles.

- Materials

Aortic intima-media removing and fractionment into microsomes and cell-sap were performed as previously described [1]. Purity of subcellular fractions was controlled by specific enzymatic determinations [5].

- Xylosyltransferase purification

The enzymatic purification was carried out by hydrophobic chromatography [6], in a first time onto hydrophobic kits [7] and then onto an octyl-agarose column (50 cm x 2.5 cm). The enzymatic activity was eluted with a linear gradient of KCl in 50 mM Tris HCl buffer at pH 7.2 from 0 to 1 M.

- Standard xylosyltransferase assays

The enzymatic preparation was concentrated on XM 300 Diaflo membranes (Amicon) before incubation. [^{14}C]-xylose incorporation was performed onto endogenous or exogenous acceptors (poly-L-serine). The acellular system of glycosylation was composed of :

- 200 µl of enzymatic preparation (2 mg/ml) in 50 mM Tris HCl buffer pH 7.2 . Protein concentrations were assayed using the Gornall's procedure [8] with bovine serum albumin as standard ;
- 10 µl of poly-L-serine (20 mg/ml) ;
- 10 µl of 1 mM $MnCl_2$;
- 10 µl UDP-[^{14}C]-xylose 94 pmol, specific activity (200 Ci/mol).

UDP-[^{14}C]-xylose integrity was controlled by chromatographic techniques as previously described [5] and the nature of labelled sugar was determined after hydrolysis by chromatography according to the procedure of Montreuil et al [9].

Incubations were performed at 37° C or 57° C and stopped either by the mixture : 4 % phosphotungstic acid 10 % T C A (C') or by chloroform/methanol (2:1, v/v). Organic extracts were carried out as described by Waechter et al. [10]. Radioactivity was measured in a Tri-Carb Packard liquid scintillation counting system using Packard M I 92.

- Incubation with exogenous phospholipids

The phospholipids and analogs used were tested for purity by thin layer chromatography, in solvent : chloroform/methanol/water (65:25:4, by vol), for 90 minutes at room temperature.

Phospholipids were dissolved in chloroform/methanol (2:1, v/v) containing 0.1 % sodium taurocholate and were dried under nitrogen in the assay tubes prior to the addition of enzymatic preparation. The mixture was homogenized in a Branson ultrasonic water-bath (Bransonic 220) for 3 minutes. Then [^{14}C]-xylose incorporation was performed under standard conditions.

RESULTS AND DISCUSSION

- Purification of the xylosyltransferase

In aortic intima-media cells, a xylosyltransferase was located in the cell-sap. The better purification of this enzyme was performed by hydrophobic

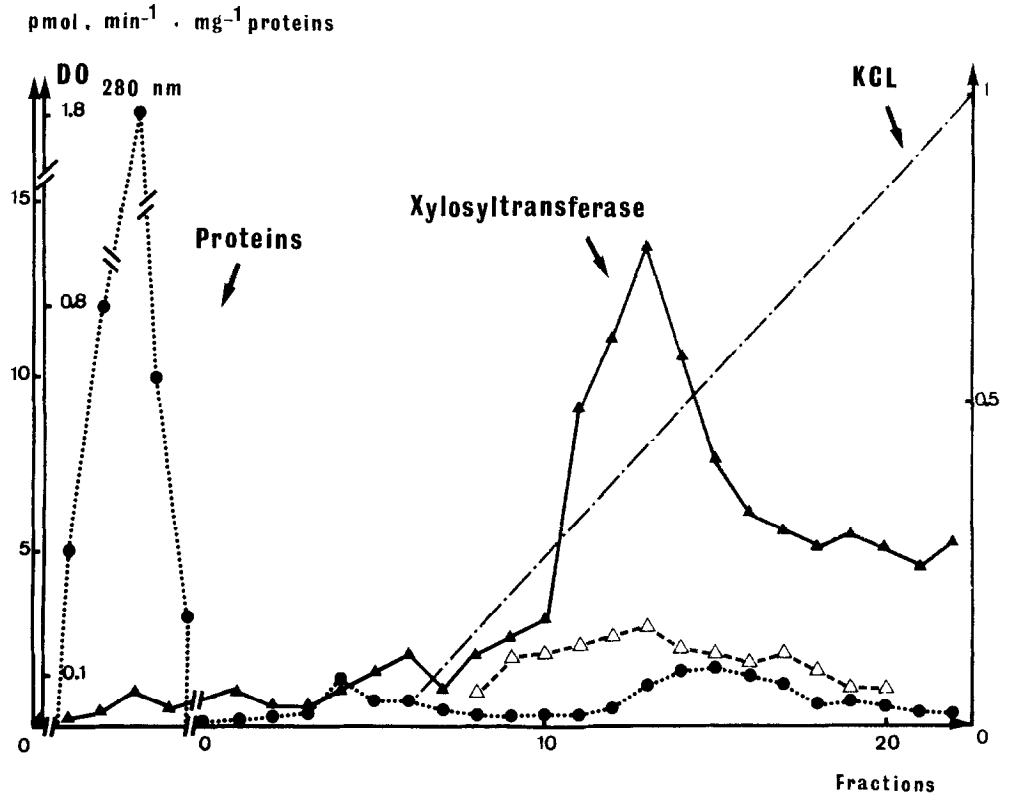

Fig. 1 - Purification of the xylosyltransferase onto octyl-agarose chromatography column.

The octyl-agarose column was prepared as described in Material and Methods. Fractions of 5 ml were collected.

●·····● proteins at 280 nm

·—·—· KCl gradient in 50 mM Tris HCl pH 7.2

△- - -△ Xylosyltransferase activity onto endogenous acceptor

▲——— Xylosyltransferase activity onto exogenous acceptor (poly-L-serine)

chromatography on an octyl-agarose column. As it was reported in fig. 1, a good binding of the xylosyltransferase was effective on octyl-agarose while almost all other proteins were not retained. This enzyme was eluted between 200 to 400 mM KCl with a maximum at 330 mM. Its purification factor was about 38 and its yield 11 %.

The labelled product (poly-L-serine-[^{14}C]-xylose) was isolated by chromatography on a G 25 Sephadex column as it was previously reported [5].

- [^{14}C]-xylose incorporation

After the purification of the xylosyltransferase by hydrophobic chromatography, enzymatical parameters were identical to those previously described for the enzymatic purification by electrofocusing [3]. The [^{14}C]-xylose incorporation onto exogenous acceptor (poly-L-serine) was maximal

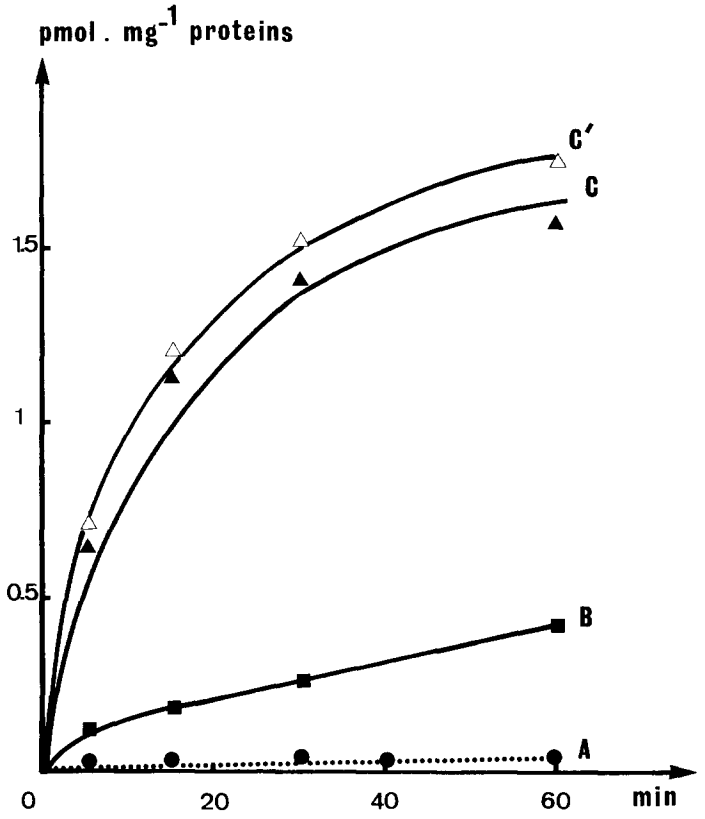

Fig. 2 - Incorporation of [^{14}C]-xylose from UDP-[^{14}C]-xylose onto poly-L-serine.

The incubations were performed at 57° C for the indicated periods in standard conditions previously described.

A : product extracted with $CHCl_3/CH_3OH$ (2:1, v/v)

B : product extracted with $CHCl_3/CH_3OH/H_2O$ (10:10:3, by vol)

C : product insoluble in water and organic solvents

C' : product precipitate by the mixture : 10 % TCA, 4 % phosphotungstic acid.

at 57° C in 50 mM Tris HCl buffer pH 7.2 in presence of 1 mM $MnCl_2$. The labelled products were isolated from 10 % TCA , 4 % phosphotungstic acid precipitate or after lipidic extractions according to their solubility in chloroform/methanol (2:1, v/v) and in chloroform/methanol/water (10:10:3, by vol) as described by Waechter et al. [10]. Results presented in fig. 2 show that [^{14}C]-xylose is principally incorporated into glycoproteins extracts obtained from precipitate C' or after lipidic extractions (C). So, no endogenous polyprenic acceptor is present in the cell-sap, moreover exogenous dolichyl phosphate does not conduct to a [^{14}C] incorporation into lipidic components extracted by chloroform/methanol (2:1, v/v) and chloroform/methanol/water (10:10:3, by vol). Therefore the biosynthesis of glycoprotein is not carried out via dolichol intermediates. Same results were obtained

by Schwartz et al. [11] for their studies on embryonic-chicken cartilage while Waechter et al. [12] have isolated dolichol-phosphate-[^{14}C]-xylose in hen oviduct.

- Effect of phospholipids on the xylosyltransferase

In an attempt to explain how phospholipids interfere on the enzymatic activity, we used a great variety of synthetic phospholipids. All these phospholipids interact with the xylosyltransferase as effectors but they do not conduct to [^{14}C]-xylose incorporation into organic extracts. Results are summarized in table I.

• The glycerol esterification either by palmitic acid (table I a), phosphorylethanolamine, phosphorylcholine or phosphorylinositol (table I b) do not affect the enzymatic activity while glycerophosphorylserine is a strong inhibitor of the xylosyltransferase. Phosphatidylserine conducts also to the same inhibition percentage while lysophosphatidylserine does not modify the enzymatic activity. Labelled products are not found in organic solvent extracts. These results confirm that xylosyltransferase is not able to catalyze the transfer of [^{14}C]-xylose onto seryl residues of phospholipids.

. Some phospholipids containing palmitic acyl residues have a marked effect on xylosyltransferase activity (table I c and I d). 1-palmitoyl-sn-glycerol-3-phosphorylcholine behaves as an inhibitor while 1,2-dipalmitoyl-sn-glycerol-3-phosphorylcholine does not interact with this enzyme, still 1-palmitoyl or 1,2-dipalmitoyl-sn-glycerol-3-phosphate interferes with a marked inhibition percentage. Therefore the presence of one or two palmitic acyl residues is not a main factor of enzymatic regulation. Moreover, a free hydroxylgroup in 2 position is without effect as similar results are obtained with lysophosphatidylcholine or phospholipids-1,3-propandiol phosphorylcholine.

. The effect of phospholipids is not related to a steric hindrance phenomenon since substitution by either one, two or three methyl groups on choline residues does not conduct to significant different results (table I d).

. We also verified the importance of the phospholipid configuration on the xylosyltransferase activity. Similar results were obtained with sn or rac phospholipids in which phosphate, phosphorylcholine or phosphoryl-ethanolamine groups were in 2 or 3 position. In the same way, identical results were obtained when palmitic acid was linked to glycerol in the 1 or 2 position for monopalmitoyl glycerol, or in the 1,2 or 1,3 positions for dipalmitoyl glycerol. Thus the interactions of phospholipids with the xylosyltransferase are not stereospecific but they are markedly related to

TABLE I

Effect of phospholipids on xylosyltransferase activity

	Experiment	Results in %
	Control	0
a	Glycerol	0 ± 2
a	1-monopalmitoyl glycerol	0 ± 5
a	1,2-dipalmitoyl glycerol	0 ± 2
a	Tripalmitoyl glycerol	0 ± 3
b	Glycerophosphoryl choline	- 10 ± 2
b	Glycerophosphoryl ethanolamine	0 ± 3
b	Glycerophosphoryl serine	- 75 ± 2
b	Glycerophosphoryl inositol	0 ± 2
c	1-palmitoyl-sn-glycerol-3-phosphate	- 90 ± 2
c	1-palmitoyl-sn-glycerol-3-phosphoryl choline	- 50 ± 3
c	1-palmitoyl-sn-glycerol-3-Lα-phosphoryl ethanolamine	- 10 ± 5
d	1,2-dipalmitoyl-sn-glycerol-3-phosphate	- 60 ± 3
d	1,2-dipalmitoyl-sn-glycerol-3-phosphoryl choline	0 ± 2
d	1,2-dipalmitoyl-sn-glycerol-3-Lα-phosphoryl ethanolamine	- 10 ± 5
d	1,2-dipalmitoyl-sn-glycerol-3-Lα, NN'-dimethyl phosphoryl ethanolamine	0 ± 3

Each reaction mixture contained 5 mM lipidic compound homogenized by sonication with the acellular system of glycosylation. Incubations were performed in optimal conditions of enzymatic activity as previously described. Results were expressed in inhibition (-) or activation (+) percentages. The control enzyme activity was 0.13 pmol/min/mg protein.

their polar headgroups. However the addition of cations such as Ca^{2+}, Na^{+} or K^{+} at 5 mM in the acellular system does not interfere on the enzymatic activity.

. The influence of fatty acyl chains was also studied. Results summarized in table II show that the inhibition percentage is higher for long fatty acyl chain (table II a) but it is not related to the insaturation

TABLE II

Influence of the nature of fatty acids involved in lysophosphatidylcholine on the xylosyltransferase activity

	Experiment	Inhibition in %
	Control	0
a	1-caprylyl-L-α-phosphorylcholine (C_8)	10 ± 5
a	1-capryl-L-α-phosphorylcholine (C_{10})	20 ± 5
a	1-lauroyl-L-α-phosphorylcholine (C_{12})	40 ± 3
a	1-myristoyl-L-α-phosphorylcholine (C_{14})	50 ± 5
a	1-palmitoyl-sn-glycerol-3-phosphorylcholine (C_{16})	60 ± 5
a	1-stearoyl-sn-glycerol-3-phosphorylcholine (C_{18})	50 ± 3
a	1-arachidonyl-glycerol-3-phosphorylcholine (C_{20})	70 ± 2
b	1-stearoyl-sn-glycerol-3-phosphorylcholine (C_{18})	50 ± 3
b	1-oleoyl-sn-glycerol-3-phosphorylcholine ($C_{18:1}$)	50 ± 5
b	1-linoleoyl-glycerol-3-phosphorylcholine ($C_{18:2}$)	50 ± 5
b	1-linolenoyl-glycerol-3-phosphorylcholine ($C_{18:3}$)	50 ± 3

Each reaction mixture contained 5 mM lysophosphatidylcholine homogenized by sonication with the acellular system of glycosylation which was composed as previously described. The control enzyme activity was 0.13 pmol/min/mg protein.

degree of fatty acids involved in lysophosphatidylcholine structures (Table II b). In order to complete this study, exogenous fatty acids were added at 5 mM in the glycosylation system, the xylosyltransferase activity is not affect by main fatty acids involved in the preceding lysophosphatidylcholine structures, excepted for lauric acid which inhibited the activity about 40 %. This last result is in agreement with the detergent properties of this product as it will be discussed further.

. Then the influence of the nature of the chemical bond (ester or ether bond) involved in lysophosphatidylcholine structures was considered. Synthetic lysophospholipids including fatty acid or alcohol with the same chain length were used. Lysophosphatidylcholine or alkyl analogs lead to a similar inhibitory effect. Therefore, the nature of the chemical bond has a weak influence on the xylosyltransferase. Identical results were obtained with analogous

structures of phosphatidic acid. Moreover, the substitution of a carboxylgroup by an hydroxylgroup has not a major influence on [^{14}C]-xylose incorporation.

. In order to elucidate the phospholipid effect on xylosyltransferase activity the various parameters involved in enzymatic reaction of glycosylation were verified :

The conductibility of the acellular system of glycosylation is unaffected by addition of exogenous phospholipids.

Integrity of UDP-[^{14}C]-xylose substrate is not modified when phospholipids are added in the glycosylation system.

In absence of the glycosyltransferase, [^{14}C]-xylose is not incorporated into phospholipids and no adsorption was found.

Ultrasonic treatment which interacts with physical properties of phospholipids unaffects the xylosyltransferase but the inhibitory effect of phospholipids such as phosphatidic acid is increased when this effector was sonicated with the enzymatic preparation. The temperatures either of sonication (16 to 40° C) or of incubation (37° C or 57° C) are without effect. Nevertheless, according to the hypothesis as the glycosyltransferase or its substrate could be enclosed into liposomes by ultrasonic treatment is excluded since addition of detergent as Triton X 100 (at 0.5 % v/v) which would lead to a liposome breaking does not interfere with phospholipid effect or enhances the inhibitory effect of phosphatidic acid. Moreover, mixed micelles between phospholipids and taurocholate could not be set forth, similar results are obtained without taurocholate in particularly with 1-palmitoylphosphatidylcholine which is soluble in water.

. As lysophosphatidylcholine is well known to have solubilization properties [13-21] the effect of several detergents was tested on the xylosyltransferase activity. They all interact as inhibitors, but this effect is higher with ionic detergents (- 60 % at 2.5 mM) than with non ionic detergents (-40 % at 3 mM). Therefore the inhibition effect of lysophosphatidylcholine could be in part related to its solubilization properties, these results are in opposite with the most studies reported upon soluble [13-14] or membranous glycosyltransferases [17-26]. In aortic wall, as the xylosyltransferase has a high molecular weight (< 300,000), it is possible that this enzymatic system can exist as an aggregate and that phospholipids or detergents prevent this aggregation which would be essential for the enzymatic activity. Thus, polar phospholipids and lysophosphatidylcholine interfere certainly with the xylosyltransferase conformation and therefore conduct to its denaturation.

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