Volume 259, number 1, 95–98 FEB 07929 December 1989

Evidence for leukotriene C_4 transport mediated by an ATP-dependent glutathione S-conjugate carrier in rat heart and liver plasma membranes

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Received 11 October 1989

Using rat heart sarcolemma and liver plasma membrane vesicles, it has been verified that the transport of leukotriene C_4 (LTC_4) across membranes is an ATP-dependent process; the apparent K_m for LTC_4 was 150 nM (heart sarcolemma) or 250 nM (liver plasma membrane). S-(2,4-dinitrophenyl)-glutathione (DNP-SG) inhibited LTC_4 uptake into the vesicles dose-dependently ($I_{50} = 25$ μM for both heart sarcolemma and liver plasma membrane vesicles). Mutual inhibition between LTC_4 and DNP-SG in uptake into the vesicles demonstrates that transport of LTC_4 is mediated by an ATP-dependent glutathione S-conjugate carrier.

Leukotriene C_4; Glutathione S-conjugate; ATP-dependent transport; Plasma membrane; (Rat heart, rat liver)

1. INTRODUCTION

Leukotrienes are a family of biologically active conjugated trienes derived from arachidonic acid via the 5-lipoxygenase pathway, and play an important role in disease processes such as local inflammation, bronchial asthma, liver injury, tissue trauma and shock ([1–5] for review). The cysteinyl leukotrienes (LTC_4, LTD_4, and LTE_4), originally known as SRS-A, increase vascular permeability and contract smooth muscles [1]. LTC_4 is formed through the reaction of LTA_4 with glutathione in various organs and cell types [5], and the reaction is catalyzed by a microsomal enzyme, leukotriene C_4 synthase, and/or isozymes of cytosolic glutathione S-transferases. LTC_4, in turn, is converted to LTD_4 and LTE_4 by two extracellular ectoenzymes, i.e. γ-glutamyltransferase and dipeptidase, respectively, whereas for this conversion LTC_4 must be precedently transported out of cells.

Recently, it has been demonstrated that transport of glutathione S-conjugate, i.e. DNP-SG, across rat liver plasma membrane as well as rat heart sarcolemma is mediated by an ATP-dependent carrier [6–9]. In the case of heart sarcolemma, the transport of DNP-SG was competitively inhibited by LTC_4 [9], and based on the result, it was hypothesized that the ATP-dependent carrier would share the translocation of LTC_4. The present study has been undertaken to examine this hypothesis using plasma membrane vesicles prepared from rat heart and liver. We now provide evidence for the first time that LTC_4, transport is an ATP-dependent process mediated by the glutathione S-conjugate carrier.

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Abbreviations: LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; DNP-SG, S-(2,4-dinitrophenyl)-glutathione; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; 4-hydroxynonenal, *trans*-4-hydroxynon-2-enal; SRS-A, slow-reacting substance of anaphylaxis; HPLC, high-performance liquid chromatography

2. MATERIALS AND METHODS

2.1. *Biochemicals*

Leukotrienes C_4, D_4 and E_4 (Salford Ultrafine Chemicals and Research Ltd, Manchester), ATP, creatine phosphate and creatine kinase (Boehringer, Mannheim), ouabain (Sigma, St. Louis), [14,15-$^3H_2$]leukotriene C_4 and [2-^{3}H]glycine-labeled GSH (New England Nuclear, Boston) were purchased from the commercial sources indicated. [^{3}H]DNP-SG was enzymatically synthesized as described previously [8,9]. Anthglutin and 4-hydroxynonenal are generous gifts from Dr T. Komai (Sankyo Co. Ltd, Tokyo, Japan) and Prof. H. Esterbauer (Graz Universität, Austria), respectively. All other chemicals were of analytical grade.

2.2. *Preparation of sarcolemma vesicles from rat heart and plasma membrane vesicles from rat liver*

Rat heart sarcolemma vesicles were prepared as described previously [8,9]. Preparation of plasma membrane (a mixture of basolateral and canalicular membranes) from rat liver was performed according to [10]. The plasma membrane-rich fraction obtained from Percoll-gradient centrifugation was suspended in 0.25 M sucrose, 0.5 mM $CaCl_2$ and 5 mM Hepes-KOH (pH 7.4), layered over 38% sucrose, and then centrifuged at 105 000 × *g* for 60 min. The resulting turbid layer at the interface was harvested, suspended in 10 ml of 0.25 M sucrose containing 10 mM Tris-HCl (pH 7.4), and centrifuged at 70 100 × *g* for 30 min. The precipitate was finally suspended in a small volume of 0.25 M sucrose containing 10 mM Tris-HCl (pH 7.4), frozen in liquid N_2, and stored at −70°C until use.

Protein was determined according to Lowry et al. [11].

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00145793/89/$3.50

2.3. *Transport of [^{3}H]LTC$_4$ into plasma membrane vesicles*

The standard incubation medium for the measurement of LTC$_4$ uptake contained membrane vesicles (50–150 μg protein), 12.5 nM [^{3}H]LTC$_4$, 0.25 M sucrose, 10 mM Tris-HCl (pH 7.4), 1 mM ATP, 100 μM anthglutin, 10 mM $MgCl_2$, 10 mM creatine phosphate and 100 μg/ml creatine kinase in a final volume of 110 μl. The incubation was carried out at 37°C under N_2 gas. Uptake of [^{3}H]LTC$_4$ into the vesicles was measured by a rapid filtration technique using Millipore filters (GVWP, 0.22-μm pore size) as described previously [8,9].

2.4. *High performance liquid chromatography*

The purity of leukotriene samples and metabolites of LTC$_4$ after incubation with membrane vesicles was analyzed by HPLC with a reverse phase column (Cosmosil 5C18, 4.6 × 250 mm, Nacalai Tesque Ltd, Kyoto) according to [12]. The mobile phase (flow rate, 0.8 ml/min) consisted of methanol/water, 7:3 (v/v), plus 0.1% acetic acid adjusted to pH 5.4 with NH_4OH.

To check the decomposition of LTC$_4$ by γ-glutamyltransferase of membrane vesicles, [^{3}H]LTC$_4$ was incubated with vesicles in the standard incubation medium at 37°C for 10 min, but no detectable reaction product was observed on HPLC.

3. RESULTS

3.1. *ATP-stimulated uptake of LTC$_4$ into heart sarcolemma and liver plasma membrane vesicles*

Fig.1 shows the time courses of LTC$_4$ uptake into the membrane vesicles. Incubation of the vesicles with 12.5 nM [^{3}LTC$_4$ in the presence of 1 mM ATP resulted in a rapid uptake of LTC$_4$, and the initial rates were 0.40 and 1.64 pmol/min per mg protein for rat heart sarcolemma and liver plasma membrane vesicles, respectively. In the control experiment, when the vesicles were incubated without ATP, heart sarcolemma vesicles took up LTC$_4$ very slowly (fig.1A), whereas liver plasma membrane vesicles showed a transient uptake of LTC$_4$ (fig.1B).

The stimulation of LTC$_4$ uptake is specific for ATP; ADP, AMP or ATP analogues, i.e. adenosine 5′-[β,γ-methylene]triphosphate and adenosine 5′-[β,γ-imido]triphosphate had no stimulatory effect. Vanadate inhibited the ATP-stimulated LTC$_4$ uptake (I_{50} = 30 μM), while ouabain and EGTA did not affect the uptake.

Fig.2 shows the effect of LTC$_4$ concentration on the rates of LTC$_4$ uptake into heart sarcolemma (A) and liver plasma membrane (B) vesicles. The uptake rate was saturated with respect to LTC$_4$ concentration. The apparent K_m value for LTC$_4$ was estimated to be 150 nM, and V_{max} was 15 pmol/min per mg protein for heart sarcolemma vesicles. Likewise, values of K_m = 250 nM for LTC$_4$ and V_{max} = 45 pmol/min per mg protein were obtained for liver plasma membrane vesicles.

3.2. *Inhibition of ATP-stimulated LTC$_4$ uptake into vesicles by glutathione S-conjugates*

ATP-stimulated LTC$_4$ uptake into heart sarcolemma and liver plasma membrane vesicles was dose-dependently inhibited by a model glutathione S-conjugate, DNP-SG (fig.3); 200 μM DNP-SG inhibited the uptake almost completely. As observed in DNP-SG uptake into rat heart sarcolemma vesicles [8], LTC$_4$ uptake into both heart sarcolemma and liver plasma membrane vesicles was effectively inhibited by glutathione S-conjugates with a long aliphatic carbon chain, e.g. S-octyl- and S-decyl-glutathione and glutathione S-conjugate of 4-hydroxynonenal (table 1). Non-radiolabeled LTC$_4$ (10 μM) suppressed [^{3}H]LTC$_4$ uptake to nearly zero, whereas LTD$_4$ and LTE$_4$ inhibited LTC$_4$ uptake by about 30%.

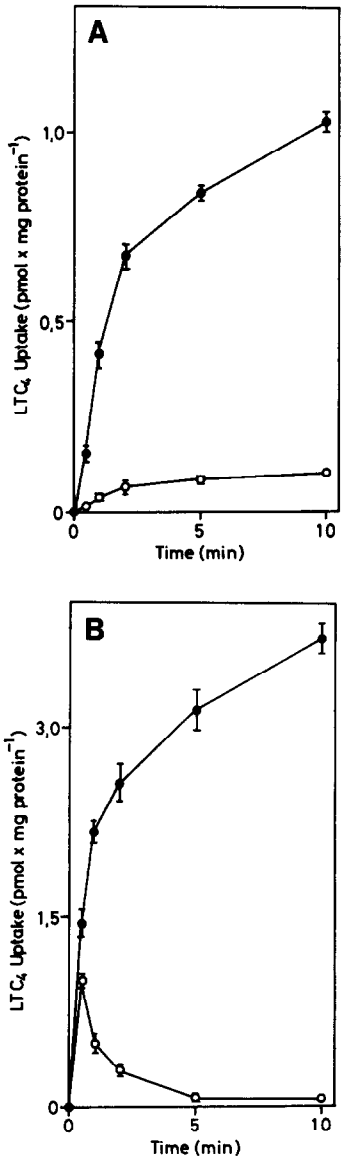

Fig.1. Time courses of LTC$_4$ uptake into heart sarcolemma (A) and liver plasma membrane (B) vesicles. Heart sarcolemma or liver plasma membrane vesicles (50 μg protein) were incubated with 12.5 nM [^{3}H]LTC$_4$ at 37°C in the absence (○) or presence (●) of 1 mM ATP in 110 μl incubation medium containing 10 mM Tris-HCl (pH 7.4), 250 mM sucrose, 10 mM creatine phosphate, creatine kinase (100 μg/ml) and 10 mM $MgCl_2$. LTC$_4$ incorporated into vesicles was determined as described in section 2. Data are expressed as mean ± SE, n = 3.

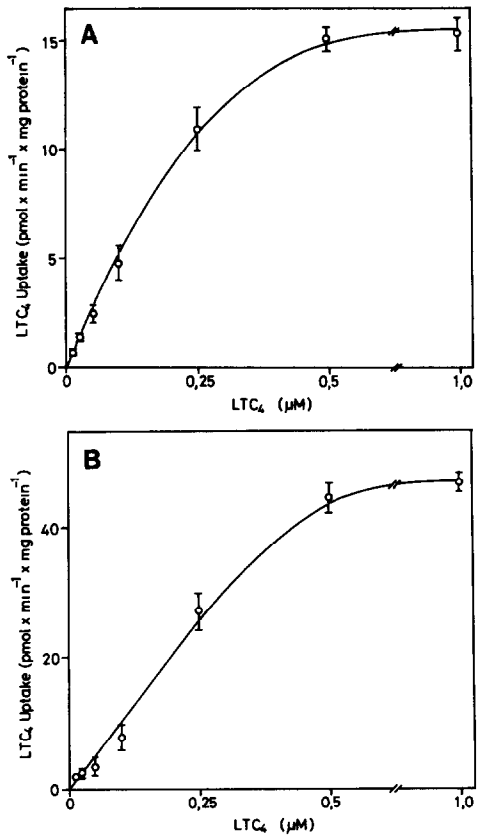

Fig.2. Effect of LTC_4 concentration on the rate of ATP-stimulated LTC_4 uptake into heart sarcolemma (A) and liver membrane (B) vesicles. Vesicles were incubated with [^{3}H]LTC_4 for 1 min at 37°C under the conditions described in fig.1. ATP-stimulated LTC_4 uptake was evaluated from the difference in radioactivity incorporated into vesicles in the presence and absence of ATP. Data are expressed as mean ± SE, $n = 3$.

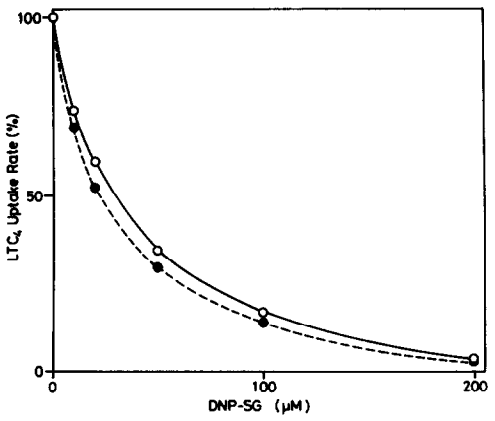

Fig.3. Effect of DNP-SG on ATP-stimulated LTC_4 uptake into heart sarcolemma (○) and liver plasma membrane (●) vesicles. Vesicles were incubated with 12.5 nM [^{3}H]LTC_4 in the presence of DNP-SG under the conditions described in fig.1. After a 1-min incubation, the reaction was terminated, and ATP-stimulated LTC_4 uptake was evaluated from the difference in radioactivity incorporated into vesicles in the presence and absence of ATP. 100% uptake rate equals 0.40 pmol/min per mg protein (heart sarcolemma) and 1.64 pmol/min per mg protein (liver plasma membrane) determined in the control experiments without DNP-SG. Data are expressed as the mean value of triplicate measurements.

Table 1

Effect of glutathione S-conjugates and cysteinyl leukotrienes on LTC_4 uptake into heart sarcolemma and liver plasma membrane vesicles

Compound	ATP-stimulated LTC_4 uptake pmol/min per mg protein (% inhibition)	
	Heart	Liver
None	0.41 (0.0)	1.64 (0.0)
S-(2,4-Dinitrophenyl)-glutathione	0.30 (27.7)	1.16 (29.5)
S-(*p*-Nitrobenzyl)-glutathione	0.31 (24.9)	1.19 (27.6)
S-(*p*-Chlorophenacyl)-glutathione	0.29 (28.6)	1.16 (29.0)
S-Methylglutathione	0.40 (2.5)	1.64 (0.2)
S-Ethylglutathione	0.39 (4.1)	1.60 (2.4)
S-Butylglutathione	0.38 (7.8)	1.51 (8.2)
S-Hexylglutathione	0.34 (17.8)	1.45 (11.3)
S-Octylglutathione	0.18 (55.3)	0.81 (50.4)
S-Decylglutathione	0.04 (89.7)	0.16 (90.4)
4-Hydroxynonenal-glutathione conjugate	0.13 (68.0)	0.48 (70.9)
Leukotriene C_4	0.01 (98.3)	0.01 (99.1)
Leukotriene D_4	0.29 (29.7)	1.11 (32.4)
Leukotriene E_4	0.31 (24.2)	1.15 (30.0)

Heart sarcolemma or liver plasma membrane vesicles (50 μg protein) were incubated with 12.5 nM [^{3}H]LTC_4 at 37°C for 1 min as described in section 2. The compounds indicated in the table were present at a concentration of 10 μM. Data are presented as mean values of triplicate measurements

4. DISCUSSION

4.1. *Transport of LTC_4 across plasma membrane is an ATP-dependent process mediated by a glutathione S-conjugate carrier*

The present study shows that transport of LTC_4 across heart sarcolemma and liver plasma membrane is an ATP-dependent process mediated by a glutathione S-conjugate carrier, which is verified by the mutual inhibition between LTC_4 and DNP-SG for the uptake into the vesicles (fig.3 and [9]) as well as by the inhibition of LTC_4 uptake by different types of glutathione S-conjugates (table 1). As compared with DNP-SG, LTC_4 is a much better substrate for the ATP-dependent glutathione S-conjugate carrier. The K_m value for LTC_4 (150 nM, heart sarcolemma; 250 nM, liver plasma membrane) is about two orders of magnitude smaller than that for DNP-SG (10–20 μM, [6–8]). At a low concentration of LTC_4 (12.5 nM) (fig.1), the rate of LTC_4 uptake into heart sarcolemma or liver plasma membrane vesicles was about 40 times higher than that of DNP-SG uptake observed with the same concentration of DNP-SG.

In the liver, cysteinyl leukotrienes and glutathione S-conjugates (e.g. DNP-SG and glutathione-conjugated bromosulfophthalein) are excreted into bile [13–17]. Biliary excretion of glutathione S-conjugates has been shown to be dependent on cytosolic ATP and not to be driven by the plasma membrane potential [18, cf. 19]. A hereditary defect in the excretion of organic anions

from hepatocytes into bile was discovered in a mutant strain of Wistar rats [20], and in the mutant rats, biliary transport of both cysteinyl leukotrienes and glutathione S-conjugate (DNP-SG) is strongly impaired, whereas hepatocellular uptake of LTC_4 from the vascular space is normal [21,22]. These findings indicate that biliary export systems for glutathione S-conjugate and cysteinyl leukotrienes are closely related or identical to each other, being in accordance with our conclusion that biliary LTC_4 transport is mediated by the ATP-dependent glutathione S-conjugate carrier.

Hepatocytes, on the other hand, are suggested to contain another specific transport system in the basolateral membrane for uptake of cysteinyl leukotrienes from the vascular space into the cells [13–15,23]. In this case, cysteinyl leukotriene uptake was not interfered by glutathione S-conjugate, S-glutathionyl-acetaminophen [23], and in this context the transport system is distinct from the ATP-dependent glutathione S-conjugate carrier discussed here.

Cardiac tissue produces LTC_4 [24] and LTC_4 release from the isolated perfused rat heart was stimulated by platelet activating factor [25]. The ATP-dependent transport system described here is likely to be responsible for the elimination of LTC_4 from heart cells and thereby facilitates further metabolism of LTC_4, namely LTD_4 and LTE_4 formation. It has been reported that some arachidonic acid metabolites including LTC_4 intracellularly modulate the G-protein-gated K^+ channel [26], indicating a physiological role of LTC_4 as an intracellular messenger. Existence of an LTC_4 degrading enzyme system in heart cells has not been elucidated. The ATP-dependent transport system may play a role in regulating intracellular LTC_4 concentrations.

4.2. *Concluding remarks*

Lam et al. have most recently reported the existence of an LTC_4 export system in human eosinophils which might be distinct from the glutathione S-conjugate carrier [27]. It is, therefore, of interest to know what type of carrier is responsible for the transport of LTC_4 in other LTC_4-producing cells, such as monocytes, mast cells, and macrophages. Isolation and characterization of the ATP-dependent glutathione S-conjugate carrier is the next important step of our study, which would facilitate our understanding of the molecular mechanism and properties of LTC_4 transport in different tissues and cell types.

Acknowledgements: Valuable discussions with Prof. Dietrich Keppler and Dr Wolfgang Hagmann (Deutsches Krebsforschungszentrum, Heidelberg, FRG) are gratefully acknowledged. Thanks are also due to Prof. Peter L.M. Jansen and Dr Ronald P.J. Oude Elferink (Academic Medical Center, Amsterdam, The Netherlands) for kindly sending articles concerning transport mutant rats. This study was supported by a Grant-in-Aid (no. 63780245 to T.I.) from the Ministry of Education, Science and Culture of Japan.

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