Volume 246, number 1,2, 177–180 FEB 06967 March 1989

Leukotriene C_4 inhibits ATP-dependent transport of glutathione *S*-conjugate across rat heart sarcolemma

Implication for leukotriene C_4 translocation mediated by glutathione *S*-conjugate carrier

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Received 29 December 1988; revised version received 7 February 1989

Sarcolemmal vesicles prepared from rat heart exhibited ATP-dependent uptake of *S*-(2,4-dinitrophenyl)glutathione (DNP-SG), which obeyed Michaelis-Menten kinetics with an apparent K_m of 21 μM for DNP-SG and a V_{max} of 0.27 nmol · 10 min^{-1} · mg $protein^{-1}$. Several model glutathione *S*-conjugates inhibited DNP-SG uptake, but leukotriene C_4 inhibited uptake much more significantly even at lower concentrations (competitive inhibition, $K_i = 1.5$ μM). However, leukotrienes D_4 and E_4, which lack the γ-glutamyl moiety, were less effective. The results suggest that the ATP-dependent transport system has a high affinity for leukotriene C_4, and may be responsible for the translocation of this compound.

Leukotriene C_4; Glutathione *S*-conjugate; ATP-dependent transport; Sarcolemma; (Rat heart)

1. INTRODUCTION

A variety of exogenous and endogenous electrophilic substances are metabolized by conjugation with glutathione either spontaneously or via catalysis by glutathione *S*-transferases (review [1]). The endogenous compounds include the epoxide leukotriene A_4 which can be converted to the glutathione derivative leukotriene C_4 [2] exhibiting smooth-muscle-contracting activity [3]. Some cytosolic glutathione *S*-transferase isozymes [4,5] and a distinct microsomal enzyme [6] are active in the reaction, and subsequently glutathione *S*-conjugates are eliminated from cells.

The existence of an energy-dependent common transport system for glutathione *S*-conjugates and glutathione disulfide (GSSG) in rat heart was reported by Ishikawa et al. [7–9]. Translocation of glutathione *S*-conjugates is an important step in inter-organ glutathione metabolism and in formation of mercapturic acid in the kidney. Metabolism of leukotriene C_4 comprises extracellular ectoenzyme-catalyzed reactions which include γ-glutamyltransferase (leukotriene D_4 formation) and dipeptidase (leukotriene E_4 formation). For this conversion, preceding translocation of leukotriene C_4 across the plasma membrane should take place. However, present knowledge concerning the molecular mechanism of translocation of this compound is limited.

This study addresses the question as to the mechanism of transport of glutathione *S*-conjugates across rat heart sarcolemmal membranes, in particular the ATP requirement. It is demonstrated for the first time that ATP-stimulated transport of glutathione *S*-conjugates is substantially inhibited by leukotriene C_4, which suggests that the glutathione *S*-conjugate transport system in heart sarcolemma may share translocation of leukotriene C_4.

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

2. MATERIALS AND METHODS

2.1. *Biochemicals*

GSSG, ATP, ADP, AMP, creatine phosphate and creatine kinase (Boehringer, Mannheim), adenosine 5′-[β,γ-methylene]-triphosphate, adenosine 5′-[β,γ-imido]triphosphate and ouabain (Sigma), leukotrienes C_4, D_4 and E_4 (Salford Ultrafine Chemicals and Research, Manchester), [2-^{3}H]glycine-labeled GSH (New England Nuclear) were purchased from the commercial sources indicated. All other chemicals were of analytical grade.

2.2. *Preparation of sarcolemmal vesicles from rat heart*

Rat heart sarcolemmal vesicles were prepared according to Kuwayama and Kanazawa [10] with some modifications. Briefly, rat heart ventricles (6 g) were homogenized in 20 ml of 0.25 M mannitol containing 70 mM Tris-H_2SO_4 (pH 7.4) and 0.1 mM *p*-aminomethylbenzenesulfonate using an Ultra Turrax at low speed. The 7000 × *g* supernatant of the homogenate was further centrifuged at 70100 × *g* for 25 min. The resulting sediment was suspended in 5 ml of 0.25 M mannitol containing 70 mM Tris-HCl (pH 7.4), and then homogenized with a Potter-Elvehjem homogenizer.

The homogenate was layered over 6 ml of 0.64 M sucrose and 20 mM imidazole-HCl (pH 7.4) and centrifuged at 70100 × *g* for 10 min using a swing-type rotor. The 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 70100 × *g* for 30 min. The precipitate was finally suspended in a small amount 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. Orientation of membrane vesicles was determined according to [11], about 50% of the population being estimated to be inside-out. Details of the procedure and characterization of the sarcolemma vesicles will be reported elsewhere (in preparation).

2.3. *Preparation of ^{3}H-labeled S-(2,4-dinitrophenyl)-glutathione (DNP-SG)*

^{3}H-labeled DNP-SG was enzymatically synthesized with radiolabeled GSH and 1-chloro-2,4-dinitrobenzene using basic isozymes of glutathione *S*-transferase prepared from rat liver [8]. DNP-SG was purified from the reaction mixture by chromatography using a QAE-Sephadex column (in preparation). The glutathione *S*-conjugate monitored for purity showed a single, sharp peak of radioactivity as well as of absorbance at 240 nm on high-performance liquid chromatography (HPLC) using a reversed-phase column (Cosmosil 5C18 4 × 250 mm, Nakalai Tesque, Kyoto) using 50% aqueous acetonitrile as eluent.

2.4. *Transport of DNP-SG into sarcolemmal vesicles*

The standard incubation medium for measurement of DNP-SG uptake contained sarcolemmal vesicles (150 μg protein), 100 μM DNP-SG, 0.25 M sucrose, 10 mM Tris-HCl (pH 7.4), 1 mM ATP, 10 mM $MgCl_2$, 10 mM creatine phosphate and 100 μg/ml creatine kinase in a final volume of 110 μl. Incubation was carried out at 37°C. Uptake of ^{3}H-labeled DNP-SG by the vesicles was assayed by the rapid filtration technique using Millipore filters (GVWP, 0.22 μm pore size). Radioactivity remaining on filters was determined in a liquid scintillation counter. To check the decomposition of DNP-SG by γ-glutamyltransferase, DNP-SG was incubated with vesicles in the standard incubation medium for 30 min. No detectable reaction product however, was observed on HPLC.

3. RESULTS AND DISCUSSION

3.1. *ATP-stimulated DNP-SG uptake by heart sarcolemma vesicles*

Previous work using isolated perfused rat heart showed the existence of a common transport system for GSSG and glutathione *S*-conjugates [7,8]. A close relationship between the transport rate and cytosolic free ATP/ADP ratio suggests energy-dependent transport of GSSG as well as glutathione *S*-conjugates across the sarcolemma [9]. Here, the ATP requirement for transport of a model glutathione *S*-conjugate, DNP-SG, was examined using sarcolemmal vesicles prepared from rat heart.

As shown in fig.1, transport of DNP-SG into the vesicles was substantially stimulated when ATP was present in the incubation medium. Throughout the experiment, ATP was continuously regenerated via the creatine phosphate/creatine kinase reaction, and the uptake rate was kept almost constant for at least 15 min. ADP and AMP as well as ATP analogues, i.e. adenosine 5′-[β,γ-methylene]triphosphate and 5′-[β,γ-imido]triphosphate, were ineffective (not shown). Hydrolysis of the γ-phosphate of ATP is suggested to be essential for the transport. On the other hand, ATP-stimulated DNP-SG uptake was affected by neither ouabain nor EGTA, indicating that the transport is independent of Na^+,K^+- and Ca^{2+}-ATPase activities.

3.2. *Inhibition of DNP-SG uptake by leukotriene C_4*

The ATP-stimulated DNP uptake was inhibited by GSSG and several glutathione *S*-conjugates, i.e. *S*-(2,4-dinitrophenyl)glutathione, *S*-(*p*-nitrobenzyl)glutathione, *S*-(*p*-chlorophenacyl)glutathione and *S*-hexylglutathione (table 1; compounds present at 100 μM in the incubation medium). The ATP-dependent transport system in rat heart sarcolemma may have a broad substrate specificity for various glutathione *S*-conjugates.

Leukotriene C_4 is a naturally occurring glutathione *S*-conjugate, which strongly inhibited the uptake of DNP-SG (fig.2). The I_{50} value was about 5 μM under these incubation conditions

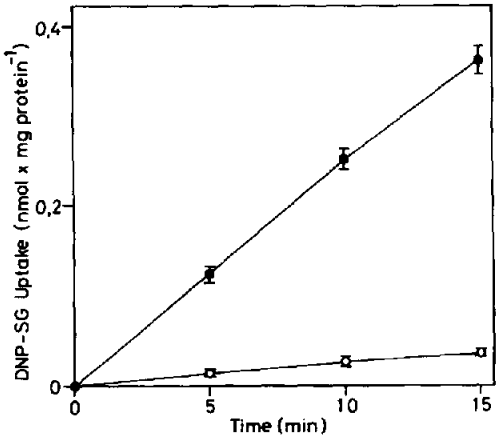

Fig.1. Time course of DNP-SG uptake by heart sarcolemmal vesicles in the absence (○) or presence (●) of 1 mM ATP. Sarcolemmal vesicles (150 μg protein) were incubated with 100 μM [^{3}H]DNP-SG at 37°C in 110 μl incubation medium containing 10 mM Tris-HCl (pH 7.4), 250 mM sucrose, 10 mM creatine phosphate, creatine kinase (100 μg/ml) and 10 mM $MgCl_2$. DNP-SG incorporated into vesicles was determined as described in section 2. Data expressed as means ± SE, n = 3.

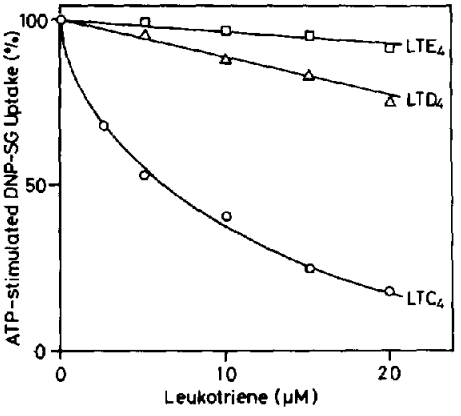

Fig.2. Effect of leukotrienes C_4, D_4 and E_4 on ATP-stimulated DNP-SG uptake by heart sarcolemmal vesicles. Sarcolemmal vesicles were incubated with 100 μM [^{3}H]DNP-SG in the presence of leukotriene C_4 (LTC_4, ○), D_4 (LTD_4, △), or E_4 (LTE_4, □) under the conditions described in fig.1. After 10 min incubation, the reaction was terminated. ATP-stimulated DNP-SG uptake was evaluated from the difference in radioactivity incorporated into vesicles in the presence and absence of ATP. 100% uptake equals 0.27 nmol·10 min^{-1}·mg protein^{-1}, determined in the control experiment without leukotriene. Data expressed as means of three different series of experiments.

([DNP-SG] 100 μM). This value is one order of magnitude lower than thoe of the above-mentioned glutathione *S*-conjugates, suggesting a significantly high affinity of the transport system for this natural glutathione *S*-conjugate. Leukotriene D_4 and E_4, which lack the γ-glutamyl moiety, have weak inhibitory effects as compared to leukotriene C_4.

Table 1

Effect of GSSG and glutathione *S*-conjugates on ATP-stimulated DNP-SG uptake by sarcolemmal vesicles

Compound	ATP-stimulated DNP-SG uptake	
	(nmol/10 min per mg protein)	(%)
None	0.272	100
GSSG	0.223	82.0
S-(*p*-Chlorophenacyl)glutathione	0.148	54.6
S-(2,4-Dinitrophenyl)glutathione	0.137	49.6
S-(*p*-Nitrobenzyl)glutathione	0.131	47.9
S-Hexylglutathione	0.101	37.3

Sarcolemmal vesicles were incubated with 100 μM [^{3}H]DNP-SG under the conditions described in fig.1. Concentration of compounds indicated was 100 μM in the incubation medium. ATP-stimulated DNP-SG uptake was calculated from the difference in radioactivities incorporated into vesicles in the presence and absence of ATP. Data are expressed as means of three different series of experiments

Fig.3 shows a Lineweaver-Burk plot of DNP-SG uptake by vesicles incubated in the presence or absence of leukotriene C_4. In the control experiment, ATP-stimulated DNP-SG uptake exhibited Michaelis-Menten kinetics with an apparent K_m of 21 μM and V_{max} of 0.27 nmol·10 min^{-1}·mg protein^{-1}. This V_{max} is less than the value which should be expected from the rate of DNP-SG release from isolated perfused heart [7]. In the presence of leukotriene C_4, DNP-SG transport was inhibited competitively; K_i was estimated to be 1.5 μM, which is much smaller than the K_m value (21 μM) obtained for DNP-SG.

3.3. *Concluding remarks*

A microsomal enzyme distinct from glutathione *S*-transferase is active in the conversion of leukotriene A_4 to C_4 [6], whereas some isozymes of cytosolic glutathione *S*-transferase, e.g. Yb_2Yb_2 and Yn_1Yn_1 in rat [4,5], are also effective. Rat heart contains the isozymes Yb_2Yb_2 and Yb_2Yn_1 as the major forms [12,13], and exhibits leukotriene C_4 synthetase activity [14]. A leukotriene C_4-like

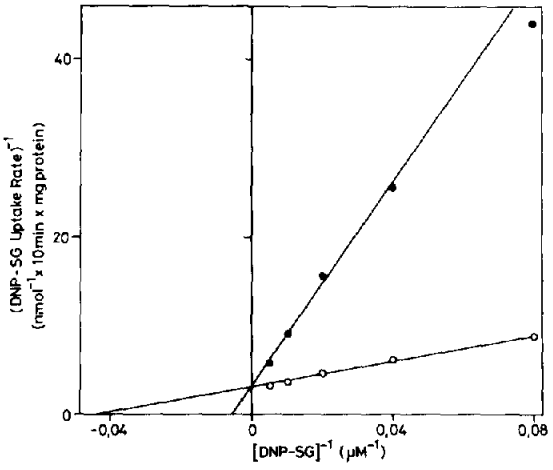

Fig.3. Lineweaver-Burk plot of ATP-stimulated DNP-SG uptake by heart sarcolemmal vesicles in the absence or presence of leukotriene C_4. Sarcolemmal vesicles were incubated with [^{3}H]DNP-SG (12.5, 25, 50, 100 and 200 μM) for 10 min in the absence (○) or presence (●) of 10 μM leukotriene C_4 under the conditions described in fig.1. Data expressed as means of three different series of experiments.

immunoreactive substance was observed in cardiac tissue treated with the calcium ionophore A23187 [15] or in myocardial cells under hypoxic conditions [16]. In isolated perfused rat hearts, leukotriene C_4 release was stimulated by platelet-activating factor [17].

The present study demonstrates that transport of glutathione *S*-conjugates across rat heart sarcolemma is an ATP-dependent process and suggests that leukotriene C_4 may be a good substrate for the ATP-dependent transport system. A similar transport system is also present in the sarcolemmal fraction prepared from bovine heart (unpublished).

Recently, ATP-dependent transport of DNP-SG has been reported in rat liver plasma membrane vesicles [18], and displays properties similar to those of the cardiac transport system reported here. As hepatobiliary elimination and subsequent enterohepatic circulation of cysteinyl leukotrienes have been demonstrated in the monkey [19] and the rat [20], it appears that the elimination of leukotriene C_4 may play an important role in the metabolism of leukotrienes, and that this might be mediated by some carrier, possibly the ATP-dependent glutathione *S*-conjugate transport system. Further studies on the transport mechanism of leukotriene C_4 and its physicochemical relevance to glutathione *S*-conjugate transport system in different organs are in progress.

Acknowledgements: The author is grateful to Professor N. Taniguchi for his encouragement and Dr K. Kobayashi for helpful discussions. 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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