Aspirin ADME

D'Eustachio, P., He, L., Huddart, R., Jassal, B., Stephan, R.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

The contents of this document may be freely copied and distributed in any media, provided the authors, plus the institutions, are credited, as stated under the terms of Creative Commons Attribution 4.0 International (CC BY 4.0) License. For more information see our license.

This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the Reactome Textbook.

15/11/2022
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references


Reactome database release: 82

This document contains 1 pathway and 26 reactions (see Table of Contents)

https://reactome.org
Aspirin ADME

Stable identifier: R-HSA-9749641

Compartments: cytosol, endoplasmic reticulum lumen, extracellular region, mitochondrial matrix

In water aspirin (acetylsalicylic acid, ASA) dissolves, dissociating into the acetylsalicylate ion (ASA-). ASA- is an anti-clotting agent and nonsteroidal anti-inflammatory drug (NSAID); the therapeutic effects are mediated through its interaction with PTGS enzymes. On a molar basis ASA- (a) is more potent as an analgesic/anti-inflammatory agent, (b) has greater gastric ulcerogenic activity, and (c) is much more effective as an inhibitor of prostaglandin biosynthesis and platelet aggregation than salicylate (ST) (Flower 1974; Mills et al, 1974; Rainsford 1975; Rainsford 1977).

Acetylsalicylic acid is only slightly soluble in conditions being found in the stomach mucosa, mostly because of unavailability of sufficient amount of solvent. The absorption, as well as the absorbing area, increases in the small intestine. Further increased absorption is achieved by dissolving tablets before ingestion or usage of ASA salts (Dressman et al, 2012). Practically 100% of therapeutic aspirin doses are taken up, mostly by intestinal mucosal cells (Artursson & Karlsson, 1991; Yee 1997).

Only a few percent of ASA- remain unchanged, the rest is hydrolyzed to salicylate (ST). The major route of ST catabolism is conjugation with glycine to form salicyluric acid. This accounts for 20–65% of the products. Conjugation to glucuronides (ester and ether) removes up to 42% of ST. Finally, a minor part also gets hydroxylated by cytochromes (Hutt et al, 1986).

**Literature references**


https://reactome.org
## Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-03</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
Acetylsalicylic acid dissolves

Location: Aspirin ADME

Stable identifier: R-HSA-9757434

Type: transition

Compartments: extracellular region

Aspirin (acetylsalicylic acid) is classified as "highly soluble", meaning that the highest therapeutically used single dose (1000 mg) dissolves in 250 ml of water to its ionized form acetylsalicylate (ASA-). ASA- in solution has an increased rate of uptake versus aspirin in tablet form and poses fewer risks to the stomach (Leonards, 1963; Dressman et al, 2012).

Followed by: SLC16A1:BSG cotransports monocarboxylates, H+ from extracellular region to cytosol, SLCO2B1-1 transports ASA- from extracellular region to cytosol of GI cells

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-03</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
SLC16A1:BSG cotransports monocarboxylates, H+ from extracellular region to cytosol

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-9645220

**Type:** transition

**Compartments:** plasma membrane, extracellular region, cytosol

Four members of the SLC16A gene family encode classical monocarboxylate transporters, MCT1-4. They all function as proton-dependent transporters of monocarboxylic acids such as lactate and pyruvate and ketone bodies such as acetacetate and beta-hydroxybutyrate. These processes are crucial in the regulation of energy metabolism and acid-base homeostasis.

SLC16A1 encodes MCT1, a ubiquitously expressed protein (Garcia et al. 1994). Defects in SLC16A1 are the cause of symptomatic deficiency in lactate transport (SDLT), resulting in an acidic intracellular environment and muscle degeneration (Merezhinskaya et al. 2000). Activating promotor mutations in SLC16A1 are associated with exercise-induced hyperinsulinism (EIHI), a dominantly inherited hypoglycemic disorder characterized by inappropriate insulin secretion during anaerobic exercise or on pyruvate load (Otonkoski et al. 2000). MCT1 requires the binding of a single transmembrane glycoprotein (basigin, BSG) for activity (Neuhoff et al, 2005; Halestrap 2013).

Higher uptake of dissolved acetylsalicylate (ASA-) versus neutral acid shows there are transmembrane transport processes specific to ASA- or similar ions (Leonards, 1963). For salicylate, experiments with monolayers of Caco-2 cells have shown a mixture of pH-dependent passive and active influx, and one of the participating transport proteins is SLC16A1 (MCT1) (Koljonen et al, 2008). Based on this observation, it is likely that SLC16A1 also transports ASA-.

**Preceded by:** Acetylsalicylic acid dissolves

**Followed by:** ST translocates from cytosol to extracellular region, ASA- (GI) translocates from cytosol to extracellular region, ASA- translocates from cytosol to ER lumen of GI cells, ST translocates from ER lumen to cytosol of GI cells
Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009-08-25</td>
<td>Authored, Edited</td>
<td>Jassal, B.</td>
</tr>
<tr>
<td>2009-11-12</td>
<td>Reviewed</td>
<td>He, L.</td>
</tr>
</tbody>
</table>
Higher uptake of dissolved acetylsalicylate (ASA-) versus neutral acid shows there are transmembrane transport processes specific to ASA- or similar ions (Leonards, 1963). For salicylate, experiments with monolayers of Caco-2 cells have shown a mixture of pH-dependent passive and active influx, and one of the participating transport proteins is SLCO2B1 (formerly OATP-B). SLCO2B1 isoform 1 is the form expressed in the small intestine (Neuhoff et al, 2005; Koljonen et al, 2008). Based on this observation, it is likely that SLCO2B1 also transports ASA-.

Preceded by: Acetylsalicylic acid dissolves

Followed by: ASA- (GI) translocates from cytosol to extracellular region, ASA- translocates from cytosol to ER lumen of GI cells, ST translocates from ER lumen to cytosol of GI cells

Literature references


ASA- translocates from cytosol to ER lumen of GI cells

Location: Aspirin ADME

Stable identifier: R-HSA-9757438

Type: uncertain

Compartments: endoplasmic reticulum lumen, cytosol

Acetylsalicylate (ASA-) diffuses into the ER lumen of GI cells. While several reactions and processes are compartmentalized in the ER lumen most small molecules can diffuse through the ER membrane (Csala et al, 2006).

Preceded by: SLC16A1:BSG cotransports monocarboxylates, H+ from extracellular region to cytosol, SLCO2B1-1 transports ASA- from extracellular region to cytosol of GI cells

Followed by: CES2 hydrolyzes ASA-

Literature references

CES2 hydrolyzes ASA-

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-9749647

**Type:** transition

**Compartments:** endoplasmic reticulum lumen

CES2 hydrolyzes acetylsalicylate (ASA-) in GI cells. Although ASA- is also hydrolyzed to salicylate (ST) without enzymatic assistance, this process is rather slow (Rowland et al, 1972; Dressman et al, 2012). In gastrointestinal mucosa cells the predominant esterase is carboxylesterase 2 (CES2) (Imai et al, 2006), and in liver it has weak activity. In liver the main aspirin esterase is CES1 (Inoue et al, 1980; Imai et al, 2006). Both enzymes hydrolyze ASA-, producing acetate and ST (Tang et al, 2006; Imai, 2006; Lian et al, 2017).

**Preceded by:** ASA- translocates from cytosol to ER lumen of GI cells

**Followed by:** ST translocates from ER lumen to cytosol of GI cells

**Literature references**


## Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-03</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
Salicylate (ST) diffuses out of the ER lumen of GI cells. While several reactions and processes are compartmentalized in the ER lumen most small molecules can diffuse through the ER membrane (Csala et al, 2006).

**Preceded by:** CES2 hydrolyzes ASA-, SLC16A1:BSG cotransports monocarboxylates, H+ from extracellular region to cytosol, SLCO2B1-1 transports ASA- from extracellular region to cytosol of GI cells

**Followed by:** ST translocates from cytosol to extracellular region, ASA- (GI) translocates from cytosol to extracellular region

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-05</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
ASA- (GI) translocates from cytosol to extracellular region

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-9757441

**Type:** uncertain

**Compartments:** extracellular region, cytosol

It is not known how acetylsalicylate (ASA-) localizes out of gastrointestinal cells. In particular, the ABCB1 efflux pump does not transport ASA- (Singh et al, 2020).

**Preceded by:** SLC16A1:BSG cotransports monocarboxylates, H+ from extracellular region to cytosol, SLCO2B1-1 transports ASA- from extracellular region to cytosol of GI cells, ST translocates from ER lumen to cytosol of GI cells

**Followed by:** ASA- acetylates ALB, BCHE hydrolyzes ASA-, SLC22A7 transports ASA-,ST from extracellular region to cytosol of hepatocytes

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-03</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
It is not known how salicylate (ST) localizes out of gastrointestinal cells. In particular, the ABCB1 efflux pump does not transport ASA- (Singh et al, 2020).

**Preceded by:** SLC16A1:BSG cotransports monocarboxylates, H+ from extracellular region to cytosol, ST translocates from ER lumen to cytosol of GI cells

**Followed by:** ALB binds ST, SLC22A7 transports ASA-,ST from extracellular region to cytosol of hepatocytes

**Literature references**

**BCHE hydrolyzes ASA-**

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-9749609

**Type:** transition

**Compartments:** extracellular region

Acetylsalicylate (ASA-) is hydrolyzed by two plasma esterases. About 80% of aspirin esterase activity in the blood is due to the erythrocyte enzyme butyrylcholine esterase (pseudocholinesterase, BCHE). BCHE hydrolyzes acetylsalicylate (ASA-), producing acetate and salicylate (Rainsford et al, 1980; Costello & Green, 1983).

**Preceded by:** ASA- (GI) translocates from cytosol to extracellular region

**Followed by:** ALB binds ST, SLC22A7 transports ASA-,ST from extracellular region to cytosol of hepatocytes

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-03</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
ASA- acetylates ALB

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-9749590

**Type:** transition

**Compartments:** extracellular region

Acetylsalicylate (ASA-) acetylates several lysine residues on albumin, which subsequently release their acetate over a long time span. This pseudo-esterase activity contributes to overall aspirin esterase activity in blood plasma (Hawkins et al, 1969; Liyasova et al, 2010).

**Preceded by:** ASA- (GI) translocates from cytosol to extracellular region

**Followed by:** ALB binds ST

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-03</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
SLC22A7 transports ASA-, ST from extracellular region to cytosol of hepatocytes

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-9749583

**Type:** transition

**Compartments:** extracellular region, cytosol

SLC22A7 is a sodium-independent multispecific organic anion transporter, expressed in liver and kidney, that also has acetylsalicylate (ASA-) and salicylate (ST) as substrate (Sekine et al, 1998; Sun et al, 2001). Uptake of ASA- by rat hepatocytes is slow and caused by an apparently linear process (Iwamoto et al, 1984).

**Preceded by:** BCHE hydrolyzes ASA-, ST translocates from cytosol to extracellular region, ASA- (GI) translocates from cytosol to extracellular region

**Followed by:** ASA-, ST translocates from cytosol to ER lumen of hepatocytes, ST translocates from cytosol to mitochondrial matrix of hepatocytes

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-03</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>

https://reactome.org
Acetylsalicylate (ASA-) and salicylate (ST) diffuse into the ER lumen of hepatic cells. While several reactions and processes are compartmentalized in the ER lumen most small molecules can diffuse through the ER membrane (Csala et al, 2006).

**Preceded by:** SLC22A7 transports ASA-,ST from extracellular region to cytosol of hepatocytes

**Followed by:** UGT1A6 glucuronates ST, CYP2,3 cytochromes hydroxylate ST to 2,3-DHBA, CYP2,3 cytochromes hydroxylate ST to 2,5-DHBA, Hydroxyl radicals oxidize ST to 2,3-DHBA, UGTs transfer GlcA from UDP-GlcA to O-centre substrates, CES1,CES2 hydrolyze ASA- to ST, ST translocates from cytosol to mitochondrial matrix of hepatocytes

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-04</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
CES1, CES2 hydrolyze ASA- to ST

Location: Aspirin ADME

Stable identifier: R-HSA-9749792

Type: transition

Compartments: endoplasmic reticulum lumen

CES1 and CES2 hydrolyze acetylsalicylate (ASA-) in hepatic cells. Although ASA- is also hydrolyzed to salicylate (ST) without enzymatic assistance, this process is rather slow (Rowland et al, 1972; Dressman et al, 2012). In gastrointestinal mucosa cells the predominant esterase is carboxylesterase 2 (CES2) (Imai et al, 2006), and in liver it has significant activity. In liver the main aspirin esterase is CES1, with minor presence of CES2 (Inoue et al, 1980; Imai et al, 2006; Schwer et al, 1997). Both enzymes hydrolyze ASA-, producing acetate and ST (Tang et al, 2006; Imai, 2006; Lian et al, 2017).

Preceded by: ASA-, ST translocate from cytosol to ER lumen of hepatocytes

Followed by: UGT1A6 glucuronates ST, CYP2,3 cytochromes hydroxylate ST to 2,3-DHBA, CYP2,3 cytochromes hydroxylate ST to 2,5-DHBA, Hydroxyl radicals oxidize ST to 2,3-DHBA, UGTs transfer GlcA from UDP-GlcA to O-centre substrates

Literature references


<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-17</td>
<td>Authored</td>
<td>Stephan R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan R.</td>
</tr>
</tbody>
</table>
UGTs transfer GlcA from UDP-GlcA to O-centre substrates

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-174931

**Type:** transition

**Compartments:** endoplasmic reticulum membrane, endoplasmic reticulum lumen

Typical O-centred substrates were chosen as examples for these isozymes. Many UDP-glucuronosyltransferases (UGTs) can transfer the glucuronyl moiety (GlcA) from UDP-GlcA to the O-centre functional group of many substrates, including salicylate (ST), to form 4-O-glucuronides (Casarett & Doull 1995, Babu et al. 1996; Kuehl et al, 2006).

**Preceded by:** ASA-, ST translocate from cytosol to ER lumen of hepatocytes, CES1, CES2 hydrolyze ASA- to ST

**Followed by:** ST metabolites diffuse out of ER lumen

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006-02-23</td>
<td>Authored, Edited</td>
<td>Jassal, B.</td>
</tr>
<tr>
<td>2008-05-28</td>
<td>Reviewed</td>
<td>D'Eustachio, P.</td>
</tr>
<tr>
<td>2016-10-10</td>
<td>Revised</td>
<td>Jassal, B.</td>
</tr>
<tr>
<td>2017-01-06</td>
<td>Reviewed</td>
<td>D'Eustachio, P.</td>
</tr>
</tbody>
</table>

https://reactome.org
UGT1A6 glucuronates ST

Location: Aspirin ADME

Stable identifier: R-HSA-9749977

Type: transition

Compartments: endoplasmic reticulum membrane, endoplasmic reticulum lumen

A side effect of phenolic glucuronidation of salicylate (ST) (resulting in the O-glucuronide) is acyl glucuronation. In particular UGT1A6 glucuronates ST preferentially at the carboxyl group (Hutt et al, 1986; Iyanagi, 2007; Kuehl et al, 2006).

Preceded by: ASA-, ST translocate from cytosol to ER lumen of hepatocytes, CES1,CES2 hydrolyze ASA- to ST

Followed by: ST metabolites diffuse out of ER lumen

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-05</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
CYP2,3 cytochromes hydroxylate ST to 2,3-DHBA

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-9749986

**Type:** transition

**Compartments:** endoplasmic reticulum membrane, endoplasmic reticulum lumen

2,3-Dihydroxybenzoate (2,3-DHBA) is a minor oxidation product of salicylate (ST). The reaction is catalyzed by several CYP2 and CYP3 cytochromes in the ER of hepatocytes (Grootveld & Halliwell, 1988; Bojić et al, 2015)

**Preceded by:** ASA-, ST translocate from cytosol to ER lumen of hepatocytes, CES1, CES2 hydrolyze ASA to ST

**Followed by:** ST metabolites diffuse out of ER lumen

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-05</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
Hydroxyl radicals oxidize ST to 2,3-DHBA

Location: Aspirin ADME

Stable identifier: R-HSA-9757462

Type: transition

Compartments: endoplasmic reticulum lumen

2,3-Dihydroxybenzoate (2,3-DHBA) can be formed by hydroxyl radicals attacking salicylate (ST) in animal cells (Ingelman-Sundberg et al, 1991). This result could not be confirmed in human liver microsomes using a different setup (Bojic et al, 2015)

Preceded by: ASA-,ST translocate from cytosol to ER lumen of hepatocytes, CES1,CES2 hydrolyze ASA- to ST

Followed by: ST metabolites diffuse out of ER lumen

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-04</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
2,5-Dihydroxybenzoate (gentisic acid, 2,5-DHBA) is a minor oxidation product of salicylate (ST). The reaction is catalyzed by several CYP2 and CYP3 cytochromes in the ER of hepatocytes (Grootveld & Halliwell, 1988; Bojic et al, 2015).

**Preceded by:** ASA-,ST translocate from cytosol to ER lumen of hepatocytes, CES1,CES2 hydrolyze ASA- to ST

**Followed by:** ST metabolites diffuse out of ER lumen, 2,5-DHBA translocates from ER lumen to mitochondrial matrix

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-17</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
ST translocates from cytosol to mitochondrial matrix of hepatocytes

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-9757454

**Type:** uncertain

**Compartments:** mitochondrial matrix, cytosol

It is not known how salicylate (ST) translocates to the mitochondrial matrix.

**Preceded by:** ASA-,ST translocate from cytosol to ER lumen of hepatocytes, SLC22A7 transports ASA-,ST from extracellular region to cytosol of hepatocytes

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-04</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
2,5-DHBA translocates from ER lumen to mitochondrial matrix

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-9757456

**Type:** uncertain

**Compartments:** endoplasmic reticulum lumen, mitochondrial matrix

It is not known how 2,5-DHBA translocates to the mitochondrial matrix.

**Preceded by:** CYP2,3 cytochromes hydroxylate ST to 2,5-DHBA

**Followed by:** ACSM2B-like proteins transform 2,5-DHBA to 2,5-DHB-CoA

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-04</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
ACSM2B-like proteins transform 2,5-DHBA to 2,5-DHB-CoA

Location: Aspirin ADME

Stable identifier: R-HSA-9749971

Type: transition

Compartments: mitochondrial matrix

ACSM2B-like enzymes catalyze the transfer of acyl groups onto CoA (Vessey et al., 1976). This is a necessary step for conjugation of 2,5-DHBA with glycine (Wilson et al., 1978).

Preceded by: 2,5-DHBA translocates from ER lumen to mitochondrial matrix

Followed by: GLYAT-like proteins transfer glycine to 2,5-DHB-CoA to form gentisuric acid

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-17</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
GLYAT-like proteins transfer glycine to 2,5-DHB-CoA to form gentisuric acid

Location: Aspirin ADME
Stable identifier: R-HSA-9750001
Type: transition
Compartments: mitochondrial matrix

GLYAT-like enzymes catalyze the transfer of acyl groups from acyl-CoA onto glycine (Webster et al., 1976). Conjugation of 2,5-DHBA with glycine yields gentisuric acid which is detected after aspirin intake (Wilson et al., 1978).

Preceded by: ACSM2B-like proteins transform 2,5-DHBA to 2,5-DHB-CoA
Followed by: Glycine conjugates translocates from mitochondrial matrix to cytosol

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-09</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>

https://reactome.org
ST metabolites diffuse out of ER lumen

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-9757459

**Type:** uncertain

**Compartments:** endoplasmic reticulum lumen, cytosol

While several reactions and processes are compartmentalized in the ER lumen most small molecules can diffuse through the ER membrane (Csala et al, 2006).

**Preceded by:** UGT1A6 glucuronates ST, CYP2,3 cytochromes hydroxylate ST to 2,3-DHBA, CYP2,3 cytochromes hydroxylate ST to 2,5-DHBA, Hydroxyl radicals oxidize ST to 2,3-DHBA, UGTs transfer GlcA from UDP-GlcA to O-centre substrates

**Followed by:** ABCC2, ABCC3 transport salicylate metabolites from cytosol to extracellular region of hepatic cells

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-04</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
Glycine conjugates translocates from mitochondrial matrix to cytosol

Location: Aspirin ADME

Stable identifier: R-HSA-9750660

Type: uncertain

Compartments: mitochondrial matrix, cytosol

It is not known how glycine conjugation products translocate out of the mitochondrion.

Preceded by: GLYAT-like proteins transfer glycine to 2,5-DHB-CoA to form gentisuric acid

Followed by: ABCC2, ABCC3 transport salicylate metabolites from cytosol to extracellular region of hepatic cells

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-09</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
ABCC2, ABCC3 transport salicylate metabolites from cytosol to extracellular region of hepatic cells

**Location:** Aspirin ADME

**Stable identifier:** R-HSA-9750656

**Type:** transition

**Compartments:** extracellular region, cytosol

ATP-binding cassette sub-family C members 2 and 3 (ABCC2, ABCC3) transport glucuronides out of liver and kidney cells. They also are able to transport organic anions and amino acid conjugates (Cui et al, 1999; Zelcer et al, 2001; Järvinen, 2019).

**Preceded by:** ST metabolites diffuse out of ER lumen, Glycine conjugates translocates from mitochondrial matrix to cytosol

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Year</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021-08-09</td>
<td>Authored</td>
<td>Stephan, R.</td>
</tr>
<tr>
<td>2021-10-13</td>
<td>Reviewed</td>
<td>Huddart, R.</td>
</tr>
<tr>
<td>2021-11-12</td>
<td>Edited</td>
<td>Stephan, R.</td>
</tr>
</tbody>
</table>
Salicylate binds to multiple sites on serum albumin, but at therapeutic doses only one site seems relevant, for kinetic reason. The exact binding site is unknown. The binding is reversible (Reynolds & Cluff, 1960; Borgå et al, 1976; Shen et al, 1991).

**Preceded by:** BCHE hydrolyzes ASA-, ST translocates from cytosol to extracellular region, ASA- acetylates ALB

**Literature references**


Table of Contents

Introduction 1

Aspirin ADME 2

Acetylsalicylic acid dissolves 4

SLC16A1:BSG cotransports monocarboxylates, H+ from extracellular region to cytosol 5

SLCO2B1-1 transports ASA- from extracellular region to cytosol of GI cells 7

ASA- translocates from cytosol to ER lumen of GI cells 8

CES2 hydrolyzes ASA- 9

ST translocates from ER lumen to cytosol of GI cells 11

ASA- (GI) translocates from cytosol to extracellular region 12

ST translocates from cytosol to extracellular region 13

BCHE hydrolyzes ASA- 14

ASA- acetylates ALB 15

SLC22A7 transports ASA-,ST from extracellular region to cytosol of hepatocytes 16

ASA-,ST translocates from cytosol to ER lumen of hepatocytes 17

CES1,CES2 hydrolyze ASA- to ST 18

UGTs transfer GlcA from UDP-GlcA to O-centre substrates 20

UGT1A6 glucuronates ST 21

CYP2,3 cytochromes hydroxylate ST to 2,3-DHBA 22

Hydroxyl radicals oxidize ST to 2,3-DHBA 23

CYP2,3 cytochromes hydroxylate ST to 2,5-DHBA 24

ST translocates from cytosol to mitochondrial matrix of hepatocytes 25

2,5-DHBA translocates from ER lumen to mitochondrial matrix 26

ACSM2B-like proteins transform 2,5-DHBA to 2,5-DHB-CoA 27

GLYAT-like proteins transfer glycine to 2,5-DHB-CoA to form gentisuric acid 28

ST metabolites diffuse out of ER lumen 29

Glycine conjugates translocates from mitochondrial matrix to cytosol 30

ABCC2, ABCC3 transport salicylate metabolites from cytosol to extracellular region of hepatic cells 31

ALB binds ST 32

Table of Contents 33