Reactivity of Acyl Glucuronides

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Topics

- The glucuronidation process: common drug examples
- Acyl glucuronides: Synthesis, structure-reactivity
- Case studies, migration kinetics
- *In vivo* protein adducts of diclofenac and ibuprofen
- acyl glucuronides
- Enzymatic glucuronidation including recombinant UGTs
Glucuronidation is a fundamental process in Phase II metabolism, whereby a wide range of functional groups—including those generated as primary metabolites—may be converted into highly water-soluble, readily excreted glucuronides.

[Chemical reaction image]

The cofactor UDPGA is widely distributed in the body-especially the liver

*Fifteen* UGT isoforms have been identified as responsible for human glucuronidation

Alcohols, phenols, carboxylic acids and amines can all be derivatised this way

The primary product is always the 1β-glucuronide, as shown

Some glucuronides, notably *N*-glucuronides, are not detected in pre-clinical toxicology

A few glucuronides of well-known drugs…
Morphine-6-glucuronide is a well-known example of a pharmacologically active glucuronide; the major \textit{in vivo} metabolite of morphine is the (inactive) 3-glucuronide (M3G: M6G ~ 5:1)
Preventing glucuronidation

Two case studies

• The anaesthetic propofol is significantly metabolised to its $O$-glucuronide
• This metabolism was very largely associated with the UGT1A9 isoform
• Addition of a closely related phenol, 2,5-isopropyl phenol, led to sacrificial glucuronidation of the additive:

\[
\text{Propofol} \xrightarrow{\text{UGT1A9}} \text{2,5-DIP}
\]

Recombinant human enzyme (in insect cell microsomes) was used:

*Drug Metab. Lett.* 2007, 1, 77-79.
A series of carboxylic acid derivatives was evaluated as 11β-HSD1 inhibitors. Acyl glucuronidation was a major clearance pathway for, e. g. the early lead compound AZD4017:

A detailed SAR study showed that both bicyclic and aryl carboxylic acid analogues of this series (combined with a heterocycle switch for the latter) led to greatly reduced acyl glucuronidation…

A carbamoyl glucuronide example

• As well as direct N-glucuronidation, amines can also metabolise as carbamoyl glucuronides
• These are close to acyl glucuronides in reactivity
• E. g. the antiarrhythmic agent, mexiletine:

- Glucuronidation was highly enantioselective → (R)-glucuronide
- Synthesis began with the (R)-carboxylic acid
- Unlike O-acyl glucuronides, carbamoyl glucuronides are stable to brief base hydrolysis
Enzymatic Glucuronide Synthesis

• Hepatic microsomes, recombinant human UGTs and engineered glycosynthases all offer possible routes—and may be regioselective in some cases.

• Example-species-selective glucuronidation of an oral antithrombotic candidate:

Cf. human liver microsomes: almost entirely the 3-glucuronide.

Human UGTs- site selectivity:

Mycophenolic acid- an important immunosuppressant, e.g. in transplantation:

\[ \text{Mycophenolic acid} \rightarrow \text{acyl glucuronide} \]

...here again, the acyl glucuronide retains some on-target activity.

*Drug Metab. Dispos.* 2005, **33**, 139-146.

Use of an engineered glycosynthase

- Wild type glucuronidase ex *E. coli* (EC 3.2.1.31)
- Key mutation, Glu504 → Gly/Ala/ Ser, abolishes hydrolytic activity
- A *glucuronyl fluoride* was used as the glycosyl donor
- A range of *O-alkyl glucuronides* made, generally good yields

Acyl Glucuronides
O-Acyl (ester) glucuronides are undoubtedly protein reactive. 
*Are* they toxic metabolites?

- Many well-known drugs, including NSAIDS, are significantly metabolised as acyl glucuronides—see below.
- Considering first *chemical* reactivity, the nature of R is important:
  - R = aryl: Reactivity predictable using Hammett considerations.
  - R = alkyl: Degree of α-substitution is very important…

R = CH₂R’, R = CHMeR’, R = CMe₂R’ show a clear gradation of properties.

**Ibuprofen**  **Diclofenac**  **Mycophenolic Acid**
Chemical Reactivity of Acyl Glucuronides

In addition to direct reaction with nucleophiles (hydrolysis or amination by e. g. lysine residues in proteins), acyl glucuronides may undergo acyl migration in a base-catalysed process...

- Acyl migration is rapid in vitro (e. g. aq. buffer) at pH 6.5 or greater; $t_{1/2}$ increases with the degree of $\alpha$-substitution, e. g. Me$_2$C> MeCH> CH$_2$.
- Transacylation (by external nucleophiles) is significantly slower for 2/3/4-O-acyl isomers.
- The acyl migrated species may also react with nucleophiles, e. g. Lys-NH$_2$, by addition at C(1) followed by rearrangement (glycation-Amadori pathway).
- Only $1\beta$-acyl glucuronides are good substrates for glucuronidases.
Synthesis of O-Acyl Glucuronides

Early syntheses used fully protected carbohydrate intermediates…

…only works because the AG linkage is very hindered
-Zemplen deprotection is OK

Mitsunobu synthesis

Using a fully-protected sugar:

- Synthesis of carbohydrate intermediate takes 5 steps
- Typically $\beta: \alpha = 2:1$ to $5:1$

E. g. $R =$

Mitsunobu synthesis is also effective using a glucuronate monoester…

- As above- yields β:α mixtures, ca. 2:1 to 5:1
- May require HPLC separation
- Allyl deprotection requires Pd(PPh₃)₄; may cause complications depending on functionality in R

Synthesis of O-Acyl Glucuronides: Selective Acylation

- Following the above, a general high-yielding synthesis of glucuronate monoesters is available
- E.g. benzyl ester:

Allyl and PMB esters are also useful; compatibility of functionality in R will decide which is appropriate.

Using minimal carbohydrate protection and highly selective acylation, a wide range of 1β-O-acyl glucuronides can be prepared with excellent anomeric selectivity...

This method exploits the kinetic anomeric effect.

Excellent β:α ratios (typically 98:2) result

Our optimum conditions: R’CO₂H, HATU, N-Me morpholine, MeCN
In Vitro Stability of O-Acyl Glucuronides

- NMR studies in buffer at pH 7.4 show a clear structure-reactivity profile.
- For the profens and related NSAIDs, α-substitution is key.
- Composite reaction is a mixture of transacylation and hydrolysis:

\[
\begin{align*}
\text{Acyl glucuronide} & \quad k_h \quad h^{-1} \quad t_{1/2} \quad h \\
R_1 = R_2 = H & \quad 2.353 \quad 0.29 \\
R_1 = \text{Me}, R_2 = H & \quad (2R)- 0.903 \quad 0.78 \\
R_1 = \text{H}, R_2 = \text{Me} & \quad (2S)- 0.405 \quad 1.71 \\
R_1 = R_2 = \text{Me} & \quad 0.029 \quad 23.30 \\
\end{align*}
\]

In this series, \( k_h \ll 10\% \) of \( k_t \).
To a good approximation, the first-order NMR degradation rate reflects \( k_t \).

Transition state analysis for the transacylation step gave excellent correlation with NMR data. Especially, for the monomethyl compounds, $k_d (2R) \sim 2x k_d (2S)$:

...the (2S)- isomer has to adopt the higher energy TS2. Empirically the ‘twofold rule’ was well known.
Acyl glucuronides of Ibuprofen and derivatives

- The twofold R/S difference still applies: $t_{1/2}$s are **1.8h, 3.7h** for **R/S Ibu**
- The remote isobutyl group has a substantial effect
- Esters (R= ethyl, allyl) also greatly slow the degradation:
  
  $$t_{1/2} = 7.24h \text{ (ethyl), 9.24h (allyl)}$$
- For two significant *in vivo* metabolites (R = H):
  
  $$R_1 = \text{OH}, R_2 = \text{H}, t_{1/2} = 5.03h; R_1 = \text{H}, R_2 = \text{CO}_2\text{H}, t_{1/2} = 4.80h$$
- Also the ionised carboxylate plays a part-different SAR for corresponding glucosides
- At present it is difficult to rationalise these long-range effects

**O-Acyl Glucuronides in Plasma**

Considering the ibuprofen-related series, in human plasma @ 37°C:

\[ \text{R}_1 = \text{R}_2 = \text{H}, \text{ Ibufenac} \]
\[ \text{R}_1 = \text{R}_2 = \text{H/Me}, \text{ (R/S)-Ibuprofen} \]
\[ \text{R}_1 = \text{R}_2 = \text{Me}, \text{ ‘Bibufenac’} \]

- In plasma, rates of reaction are higher for all compounds compared to buffer
- Hydrolysis now greatly predominates over transacylation
- At a concentration of 2 \( \mu \text{g/mL} \), \( t_{1/2} \) in plasma are:
  - Ibufenac: 0.27 h
  - (R)-Ibuprofen: 0.36 h
  - (S)-Ibuprofen: 0.22 h
  - Bibufenac: 5.2 h (2 \( \mu \text{g/mL} \))
  - …from 5 to 18-fold lower.

- HPLC-MS measurement necessary as NMR now impractical

Case Study- An unusually reactive O-acyl glucuronide

A candidate from a series of neutral endopeptidase inhibitors by Pfizer was withdrawn on toxicological grounds. Here the acyl glucuronide had a very short half life…

Culprit is the NH: $pK_a \sim 9.5$.

Cf. another member of the series…

Here, $pK_a$ (NH) $\sim 16$: the acyl glucuronide has $t_{1/2} \sim 50$ h.
Preparation of the acyl glucuronide required NH protection:

- The benzyloxyethyl (Bom) group has been used in peptide synthesis.
- Other N-protection (allyl, Boc, Z) not satisfactory.
- The final AG is highly stable at pH ~ 3; rapidly cyclises at < physiological pH.
The cyclic compound (glutarimide) was easily made independently:

...as expected, it reacts readily with nucleophiles:
The Lys adducts were stable at pH 7.4
The Cys adducts rapidly degraded at pH 7.4 but were stable at pH 3.0 for several hours.

The acyl glucuronide showed similar reactivity to the imide with nucleophiles including hydrolysis
...we concluded that intermediacy of the imide explained the reactivity of the AG

*J. Med. Chem.* 2007, 50, 6165-6176
Proteomics of Diclofenac Acyl Glucuronide

- Diclofenac AG has a short half-life and is known to be protein reactive \textit{in vitro}.
- We sought protein adducts of the AG in diclofenac patients \textit{without} adverse drug reactions.
- Other important diclofenac metabolites are the 4’- and 5-hydroxy derivatives:

...both are oxidised to quinoneimines which can deplete glutathione

Diclofenac quinone imine forms albumin adducts in vitro

NB: the 5-OH metabolite is the more readily oxidised; the derived QI is shown here.

RH(DCFQ)PYFYAPELFFAK

mass/charge, Da
Synthesis of diclofenac AG:

...we obtained a batch of 550 mgs, pure 1β-anomer, for the \textit{in vitro} studies.
**In vitro** protein reactivity:

- Diclofenac AG was incubated with human serum albumin (HSA) at 50:1 molar ratio.
- Of 59 Lys residues in HSA, *eight* were consistently modified.
- Both acylation and glycation were observed:
  - Acylation adducts arise principally from the starting $1\beta$-AG isomer.
  - Apparently the 2/3/4 –$O$-acyl isomers all contribute to *glycation*.
  - In human plasma, *hydrolysis* dominates—cf. ibuprofen.
**In vivo protein reactivity: Clinical plasma samples**

- Six patients took part in the study
- They had taken diclofenac @ 100-150 mg/ day for at least 1 yr.
- 1-3 h after the last tablet, single plasma samples were taken and acid-stabilised
- A total of **seven** adducted residues and **ten** modified peptides were identified after tryptic digest of HSA
- The most common modification was **transacylation** but **glycation** was also seen
- In one case, **glucuronylation** (slow, direct reaction of HSA with glucuronic acid) was observed
- We conclude that HSA adduction is not, invariably, a causation of adverse drug reactions with diclofenac
- …and diclofenac AG is *not* directly cytotoxic in hepatocytes or kidney cells

Diclofenac forms albumin *glycation* adducts in man.
Diclofenac forms albumin *acylation* adducts in man

**Acylation adduct**

\[ \text{DCF-AG-Lys}199 \text{ adducts detected in man} \]

\[ \text{LDK(DCF)C(iodo)ASLQK}^{205} \]

**Unmodified peptide**

\[ \text{[M+H]}^+ \]

\[ \text{y7} \]

\[ \text{K+277} \]

\[ \text{1111.4204} \]
Reaction of ibuprofen and related NSAIDs with human serum albumin

- Ibuprofen is a very widely-used antiinflammatory drug
- Its diastereoisomeric AGs are readily synthesised by the selective acylation method
- The related carboxylic acids with $\alpha,\alpha$-dimethyl substitution, or no substitution, were converted to their AGs for comparison

\[ \text{Ibufenac: } R_1 = R_2 = H \]
\[ (R)-\text{Ibuprofen: } R_1 = H, \ R_2 = \text{Me} \]
\[ (S)-\text{Ibuprofen: } R_1 = \text{Me}, \ R_2 = H \]
\[ \text{Bibufenac: } R_1 = R_2 = \text{Me} \]

\textbf{Ibufenac} was withdrawn on safety grounds
The AGs can react with protein(s) in two distinct ways:

- Direct mass spec of the adducts was possible without tryptic digest
- Transacylation and glycation pathways can be distinguished
- Traces of *dual* adducts were also seen
<table>
<thead>
<tr>
<th>Acyl glucuronide</th>
<th>Conc. mM</th>
<th>TA: Glyc</th>
<th>Conversion %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>&lt;5:95</td>
<td>58</td>
</tr>
<tr>
<td>1</td>
<td>5.0</td>
<td>&lt;5:95</td>
<td>59</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>60:40</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>5.0</td>
<td>61:39</td>
<td>44</td>
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<tr>
<td>3</td>
<td>0.5</td>
<td>64:36</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
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<td>63:37</td>
<td>23</td>
</tr>
<tr>
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<td>44:56</td>
<td>9</td>
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<td>5</td>
<td>0.5</td>
<td>-</td>
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</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>34:66</td>
<td>8</td>
</tr>
</tbody>
</table>

2 $R_1 = R_2 = H$
3 $R_1 = \text{Me}, R_2 = H$ (R)
4 $R_1 = H, R_2 = \text{Me}$ (S)
5 $R_1 = R_2 = \text{Me}$

*Chem. Sci. 2014, 5, 3789-3794*
Conclusions

- Significant variations in TA: Glyc ratios with $\alpha$-substitution
- Reactivity pattern of ibufenac is little different from those of R/S ibuprofen but *conversion is significantly higher*
- Reactivities of (R)/(S) ibuprofen acyl glucuronides also differ
- ‘Bibufenac’ (Me$_2$ analogue) gives much lower conversion, and mainly by TA pathway
- AG of a benzoic acid reacts almost exclusively by transacylation
- Physiological levels of HSA glucosylation do not protect HSA from reaction with AGs
Interaction of glucuronides with CYPs: Case Studies

- Increasing evidence for interactions of glucuronides with CYPs
- The glucuronides may be substrates or inhibitors
- Anionic and other highly polar substrates are accepted by CYP2C8
- Drug-drug interactions may result

E. g. hydroxylation of diclofenac AG:

Same UGT isoform (UGT2B7) effects the acyl glucuronidation of 4’-OH diclofenac and the acyl glucuronidation of diclofenac itself

Dalvie, Zhang et al., J. Med. Chem. 2017, 60, 8691-8705
The compound is used as an antiplatelet agent
Requires bioactivation to the thiol shown
The AG is a major human metabolite
-appears to form covalent adducts with CYP2C8

In summary…

- Glucuronides are ubiquitous metabolites of a wide range of OH (especially phenolic), carboxylic acid and amine substrates.
- Generally they act in a purely detoxifying role.
- However, they may show on target activity in their own right.
- **Acyl glucuronides** (AGs) are frequently found as metabolites of carboxylic acid drugs, notably the NSAIDS.
- Effective syntheses of AGs have been devised and their *in vitro* reactivity (acyl migration, transacylation, glycation) is now well understood.
- We are now able to study the **proteomics** of AG interaction with HSA.
- The long-term consequences are not yet understood.
- Certain glucuronides can be either substrates for, or inhibitors of, some CYP isoforms.
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