THE HYDROLYSIS OF BILE ACID CONJUGATES BY SELECTED FUNGI

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MICHAEL RUSSEL JOHNS

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ABSTRACT

The presence of constitutive, intracellular and extracellular enzymes catalysing the hydrolysis of glycine bile acid conjugates in the fungus *Cercospora melonis* CBS 162.60 was demonstrated by the use of cell-free systems. Shake flask and fermenter studies were undertaken to determine environmental factors favouring high free bile acid yields. Two major factors were observed to reduce such yields. These were the binding of the bile acid to the mycelium and the degradation of free bile acids to non-steroidal products by the fungus.

Whole-cell cultures of *C. melonis* exhibited poor utilisation of taurine conjugates with no concomitant production of free bile acid. Incubation of synthetic bile conjugate analogues with *C. melonis* and the use of cell-free systems suggested that this was due to two major factors: firstly, the specificity of the extracellular enzyme for α-aminocarboxylic acid conjugates and secondly, the apparent inability of taurine conjugates to gain access to a constitutive, intracellular cholanoyl taurine hydrolase. It is proposed that the poor permeability of the fungal cell membrane is responsible. Hence, the low activity of whole-cell cultures of *C. melonis* on taurine conjugates suggests that an industrial process employing the fungal hydrolysis of gall is not feasible.

Comparative studies with *Curvularia fallax* IFO 8885 showed that it possessed superior specific hydrolase activity on glyco-deoxycholic acid compared to *C. melonis*, although this is not apparent from qualitative screening.

The abilities of *C. melonis*, *Curvularia coicis* IFO 7278 and *Aspergillus ochraceus* IFO 4071 (Wilhelm) to 7α-dehydroxylate cholic acid and its natural conjugates were investigated. Despite the presence of an apparently constitutive, intra-cellular 7α-hydroxycholanoyl dehydroxylase in these organisms, only low yields of dehydroxylated products were obtained with whole-cell cultures.

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BILE ACID NOMENCLATURE

The trivial names used for bile acids are given, followed by the abbreviations employed, in brackets, and their I.U.P.A.C. systematic chemical names (I.U.P.A.C.-I.U.B., 1969).

Cholic acid (CA) = 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid.

Chenodeoxycholic acid = 3α,7α-dihydroxy-5β-cholan-24-oic acid.

Dehydrocholic acid = 3α,7α,12α-trioxo-5β-cholan-24-oic acid.

Deoxycholic acid (DC) = 3α,12α-dihydroxy-5β-cholan-24-oic acid.

Lithocholic acid = 3α-hydroxy-5β-cholan-24-oic acid.

N-(α-alano)-deoxycholic acid (α-AD) = 3α,12α-dihydroxy-5β-cholan-24-oyl-α-alanine.

N-(β-alano)-deoxycholic acid (β-AD) = 3α,12α-dihydroxy-5β-cholan-24-oyl-β-alanine.

N-(α-aminomethanesulphonyl)-deoxycholic acid (Na-α-AMSD) = 3α,12α-dihydroxy-5β-cholan-24-oyl-α-aminomethanesulphonic acid.

Glycocholic acid (GC) = 3α,7α,12α-trihydroxy-5β-cholan-24-oylglycine.

Glycochenodeoxycholic acid = 3α,7α-dihydroxy-5β-cholan-24-oylglycine.

Glycodeoxycholic acid (GD) = 3α,12α-dihydroxy-5β-cholan-24-oylglycine.

Taurocholic acid (NaTC) = 3α,7α,12α-trihydroxy-5β-cholan-24-oyltaurine.

Taurochenodeoxycholic acid = 3α,7α-dihydroxy-5β-cholan-24-oyltaurine.

Taurodeoxycholic acid (NaTD) = 3α,12α-dihydroxy-5β-cholan-24-oyltaurine.

The term "free bile acid" denotes a bile acid with an unsubstituted C-24 carboxylic acid group. Sulphonic acid conjugates will be usually referred to as the sodium salt.

ABBREVIATIONS

Abbreviations of units:

amu	atomic mass units
^{o}C	degrees Celsius
d	day
g	gram
x g	gravitational acceleration (ms^{-2})
h	hour
l	litre
m	metre
M	mole per litre
m/e	mass: charge ratio
min	minute
mmol	millimole per litre
Pa	pascal (Newton per square metre)
psi	pound per square inch
rpm	revolutions per minute

Other abbreviations:

ACC	Akers Culture Collection of Imperial Chemical Industries Ltd.
ATCC	American Type Culture Collection
calcd.	calculated
CBS	Centraalbureau voor Schimmelcultures
D.O.	Dissolved oxygen
hplc	high performance liquid chromatography
HUT	Hiroshima University, Faculty of Engineering
8HQ	8-Hydroxyquinoline
I.D.	Internal diameter
IFO	Institute for Fermentation, Osaka
IMI	Commonwealth Mycological Institute
IR	Infra-red
Lit.	Literature
m.p.	melting point
R_f	Tlc mobility of a compound relative to the solvent front mobility
tlc	thin layer chromatography