Biochemical Pharmacology 86 (2013) 3–14

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Review

Methods for oxysterol analysis: Past, present and future

William J. Griffiths *, Peter J. Crick, Yuqin Wang

Institute of Mass Spectrometry, College of Medicine, Grove Building, Swansea University, Singleton Park, Swansea SA2 8PP, UK

ARTICLE INFO

Article history:
Received 4 January 2013
Accepted 28 January 2013
Available online 5 February 2013

Keywords:
Autoxidation
Cholesterol
Sterol
Cholestenoic acid
Derivatisation
Mass spectrometry

ABSTRACT

Oxysterols are oxidised forms of cholesterol or its precursors. In this article we will concentrate specifically on those formed in mammalian systems. Oxidation may be catalysed by endogenous enzymes or through reactive oxygen species forming a myriad of potential products. A number of these products are biologically active, and oxysterols may have roles in cholesterol homeostasis, neurogenesis, protein prenylation and in the immune system. Oxysterols are also implicated in aetiology of disease states including atherosclerosis, neurodegenerative and inflammatory diseases. Reports indicating the levels of oxysterols in plasma, cerebrospinal fluid and various tissues are in many cases unrealistic owing to a lack of attention to the possibility of autoxidation, a process by which oxysterols are formed from cholesterol by oxygen in air. This article comprises a critical assessment of the technical difficulties of oxysterol analysis, highlights methodologies utilising best practise and discusses newer procedures.

1. Introduction

Oxysterols are oxygenated forms of cholesterol or its precursors [1,2]. They may be formed enzymatically, via reactive oxygen species (ROS), or as artefacts of sample preparation and storage (Fig. 1). Oxyphytosterols are related products formed from sterols of the plant kingdom [3]. Oxysterol research dates back to at least the 1940s when Bergström and Wintersteiner studied cholesterol metabolites found in biofluids [4,5]. Even at that time they realised the problem of autoxidation of cholesterol and pointed out that "position 7 in cholesterol is extremely susceptible to attack by molecular oxygen" and that autoxidation products of cholesterol can be formed during isolation procedures [4,5] (Fig. 1B). Later work by Smith further highlighted the ease of autoxidation of cholesterol [6], and Schroepfer's group suggested that much of the 7β-hydroxycholesterol, 7-oxocholesterol, cholestane-3β,5α,6β-triol, 5,6-epoxycholesterols and 25-hydroxycholesterol identified in biological samples are likely autoxidation products of cholesterol [7]. It should be pointed out that 25-hydroxycholesterol, 7-oxocholesterol and 7β-hydroxycholesterol are also formed enzymatically [8]. These works illustrate the overriding problem in oxysterol research which is differentiating those oxysterols formed endogenously from those generated during storage and sample preparation. Even today, in the modern era of gas chromatography (GC)–and liquid chromatography (LC)–mass spectrometry (MS), a number of studies are published which show the tell-tale marks of autoxidation [9–11].

In recent years there has been a growing interest in oxysterol research. This is on account of a number of bio-activities of oxysterols, which include "fine-tuning" of cholesterol homeostasis through binding to the protein INSIG (insulin induced gene) and through its binding to SCAP (SREBP cleavage activating protein) there-by modulating the cleavage of SREBP-2 (sterol regulatory element binding protein 2) to its active form as the master transcription factor for the cholesterol biosynthetic pathway [12,13]. Other properties of oxysterols include being agonists or inverse agonists to nuclear receptors, e.g. to the liver X receptors (LXRs) α and β [14,15] and the retinoic acid receptor-related orphan receptors (ROR) α and γ [16], respectively, at least in *vitro*, and as ligands to G-protein coupled receptors, e.g. Epstein–Barr virus induced gene 2 (EBI2) [17,18]. Oxysterols also have potent effects in the immune system that include suppressing the production of IgA by B cells, directing the migration of activated B cells in the germinal follicle, and controlling the differentiation of monocytes into macrophages [19], and an antiviral role via macrophage production of 25-hydroxycholesterol as a component of the sterol metabolic network linked to the interferon response via Stat1 [20]. Oxysterols also have potential as markers of neurodegenerative diseases [21,22], including Alzheimer's disease, and are recently reported to be important for neurogenesis in developing brain [23].

Oxysterols are present in biological samples against a high excess of cholesterol; usually there is at least 10^3 times more cholesterol than any oxysterol. This makes oxysterol analysis challenging. The challenge is evident from the early studies of

* Corresponding author. Tel.: +44 1792 295562; fax: +44 1792 295554.
E-mail address: w.j.griffiths@swansea.ac.uk (W.J. Griffiths).

0006-2952/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bcp.2013.01.027

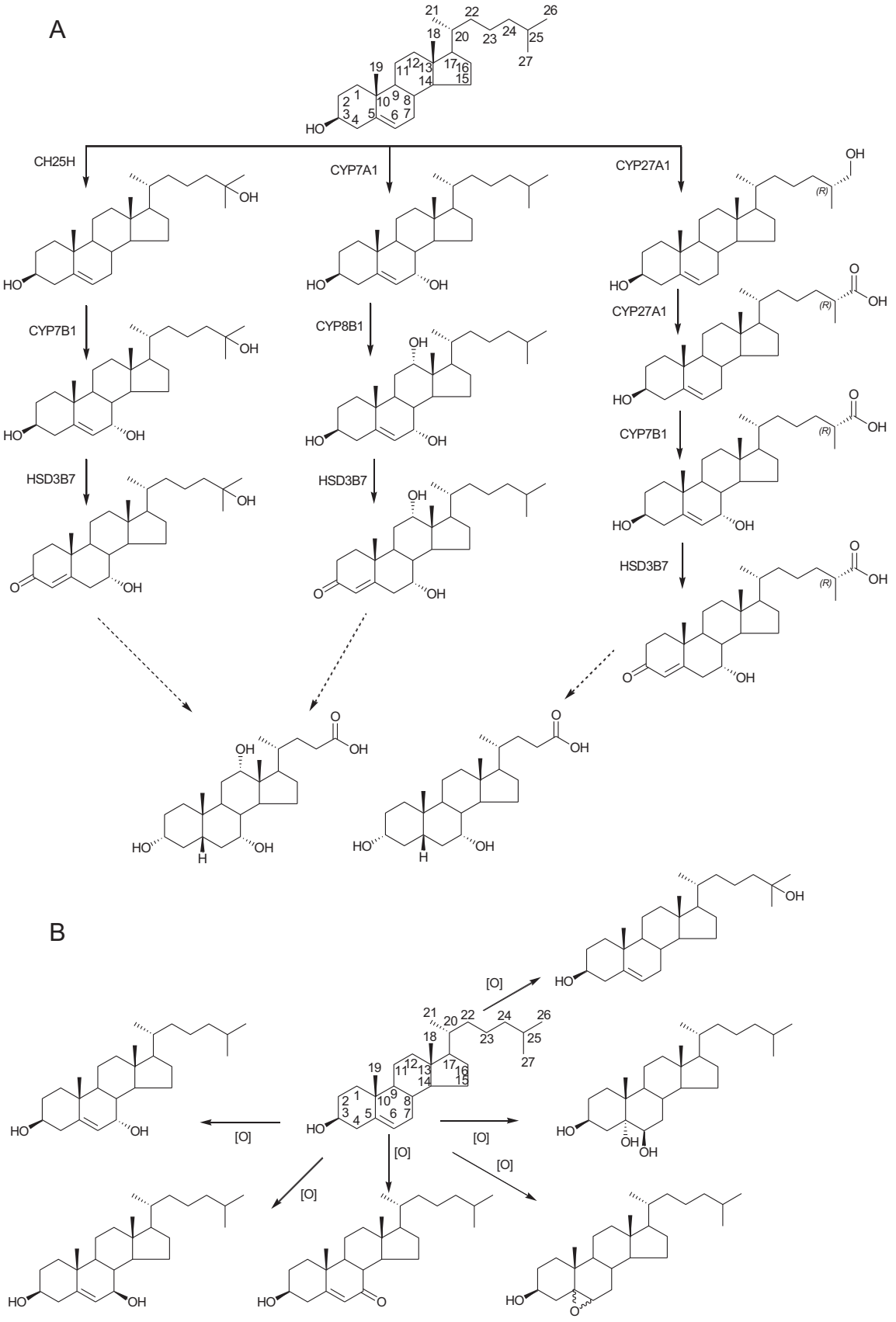

Fig. 1. (A) Structures of some of the enzymatically formed oxysterols found in plasma which are intermediates in bile acid biosynthetic pathways. Enzymes involved in oxysterol bio-transformations are show. CH25H, cholesterol 25-hydroxylase; HSD3B7, 3 beta-hydroxysteroid dehydrogenase type 7; CYP, cytochromes P450. Multiple enzymes (not shown) are involved in A-ring reduction/saturation and side-chain cleavage to give the primary bile acids. These processes are indicated by broken arrows. (B) Structures of some oxysterols formed via autoxidation. 25-Hydroxycholesterol, 7α-hydroxycholesterol and 7β-hydroxycholesterol can also be formed enzymatically.