Molecular and Cellular Endocrinology 441 (2017) 62–67

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Review

# Electrochemistry of cytochrome P450 17α-hydroxylase/17,20-lyase (P450c17)

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ARTICLE INFO

*Article history:*
Received 27 June 2016
Received in revised form
14 September 2016
Accepted 30 September 2016
Available online 3 October 2016

*Keywords:*
Steroidogenesis
Cytochrome P450c17
Androgen
Electron transfer
Mechanism
Direct electrochemistry
CYP17A1
Adrenal cortex

ABSTRACT

Within the superfamily of cytochrome P450 enzymes (P450s), there is a small class which is functionally employed for steroid biosynthesis. The enzymes in this class appear to have a small active site to accommodate the steroid substrates specifically and snuggly, prior to the redox transformation or hydroxylation to form a product. Cytochrome P450c17 is one of these and is also a multi-functional P450, with two activities, the first 17α-hydroxylation of pregnenolone is followed by a subsequent 17,20-lyase transformation to dehydroepiandrosterone (DHEA) as the dominant pathways to cortisol precursors or androgens in humans, respectively. How P450c17 regulates these two redox reactions is of special interest. There is a paucity of direct electrochemical studies on steroidogenic P450s, and in this mini-review we provide an overview of these studies with P450c17. Historical consideration as to the difficulties in obtaining reliable electrochemistry due to issues of handling proteins on an electrode, together with advances in the electrochemical techniques are addressed. Recent work using Fourier transformed alternating current voltammetry is highlighted as this technique can provide both catalytic information simultaneously with the underlying redox transfer with the P450 haem.



## 1. Introduction

The ability of cytochrome P450 enzymes (P450s) to activate molecular oxygen and catalyse a wide variety of chemical transformations with outstanding regio- and stereoselectivity (Yoshimoto and Auchus, 2015) has drawn major interest to their application in biotechnology, pharmaceutics and medicine (Bernhardt and Urlacher, 2014, Bistolas et al., 2005, Shumyantseva et al., 2005). The exploitation of the P450's catalytic function offers unprecedented possibilities for biotechnology applications, if the electron transfer chain could be replaced by a mediated or preferably a direct electron transfer from a solid state electrode to the protein. Motivation to integrate redox proteins into an electrical circuit has both fundamental and applied value. Although many electrochemical studies on P450s have been reported so far, there are several challenges to be understood and overcome to obtain useful electrochemical data for this class of enzymes. However, there has been a number of electrochemical studies using direct electrochemistry of proteins and an interested reader is encouraged to consult several reviews describing the challenges as well as related examples: (i) immobilisation or "wiring" of the protein to the electrode surface (Bistolas et al., 2005, Schneider and Clark, 2013, Shumyantseva et al., 2005); (ii) maintaining the protein's activity and control over regulatory processes (Bernhardt and Urlacher, 2014, Lundemo and Woodley, 2015, Sadeghi et al., 2011); and (iii) understanding the electron transfer kinetics between molecules (Fleming et al., 2006, Léger and Bertrand, 2008).

Cytochrome P450c17 (or P450c17, P450 17A1, CYP17A1) is a member of the cytochrome P450 super family and, as such, employs a haem cofactor to activate molecular oxygen whereby one

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http://dx.doi.org/10.1016/j.mce.2016.09.034
0303-7207/

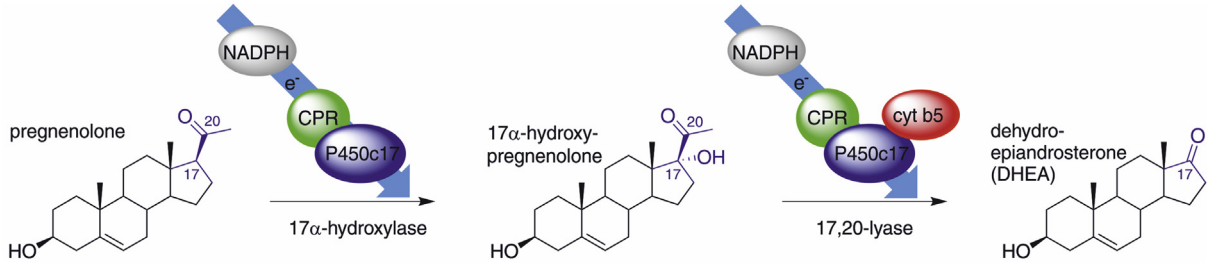


**Fig. 1.** P450c17 catalyses important steps in the steroid synthesis through multiple hydroxylations. Required electrons are delivered through cytochrome P450 oxidoreductase (CPR). To study, understand and exploit the thermodynamics and kinetics of charge transfer reactions is the playground of electrochemistry and, thus, it can provide insights into the mechanistic aspects of the P450c17 catalysis.

oxygen atom is inserted into a C-H bond to perform a hydroxylation, and the other oxygen atom is reduced and eliminated as water (Porubek, 2013). P450c17 is crucially involved in steroidogenesis, catalysing the formation of endogenous androgens and glucocorticoids and represents a branching point in the steroid pathway between glucocorticoids and androgen production. P450c17 is one of only three P450s in the steroidogenic pathway (including P450 aromatase and P450 side chain cleavage) known to be involved in concerted multiple reactions resulting in cleavage of a side-chain or a lyase reaction. Predominately in humans, this reaction involves the 17α-hydroxylation of pregnenolone to 17α-hydroxypregnenolone and further 17,20-lyase reaction to produce dehydroepiandrosterone (DHEA) (see Fig. 1). The mechanism of the 17,20-lyase reaction remains unknown. However, the second hydroxylation results in an unstable intermediate which collapses by cleaving the C17-C20 bond. The most likely mechanism proceeding from compound I involves abstraction of the 17-hydroxyl hydrogen atom followed by radical fragmentation and recombination (Yoshimoto et al., 2016). An alternative mechanism has been proposed, involving nucleophilic attack of the ferric peroxide anion on C-20 (Akhtar and Wright, 2015, Gregory et al., 2013).

P450c17 is a microsomal P450 enzyme which is expressed in the adrenal cortex and the gonads. Both hydroxylation reactions are redox-based and each begins with two electrons from NADPH transferred via cytochrome P450 oxidoreductase (CPR, also POR, CYPOR, CYP450R) to the P450c17 (haem) leading to reduction of molecular oxygen and insertion of an oxygen atom into the steroid. Interestingly, an allosteric interaction by cytochrome b5 (cyt b5) drives the P450c17 towards the 17,20-lyase reaction of P450c17 and synthesis of androgen precursors. As demonstrated recently, the latter affects the kinetics of electron movement in P450c17 (Simonov et al., 2015), that could be an evolutionary mechanism for sequential catalytic transformations of a substrate.

This review focusses on the direct electrochemistry of P450c17 (Estabrook et al., 1996, Johnson et al., 2006, Shumyantseva et al., 2011, Simonov et al., 2015, Yoshimoto and Auchus, 2015). Since P450c17 is membrane anchored, as is CPR, there are significant challenges in obtaining meaningful data which relate to how P450c17 functions in the physiological environment. Importantly, this review illustrates how the technical advances in electrochemistry and materials science have enabled new insight and understanding of redox-driven electronic behaviour of P450c17 in a biomimetic environment.

## 2. Biological considerations

Electrochemical investigation of redox proteins offers enormous challenges which have restricted studies and limited the data achievable as reviewed for P450s (Bond et al., 2007). Conventional non-protein electrochemical studies use milli-molar concentrations, thus for protein studies to achieve these amounts usually require efficient recombinant expression of milligrams of protein for investigation. Interference by oxygen reduction processes requires anaerobic conditions, which for proteins can result in foaming and denaturation. Unless the protein is especially stable, metal based electrodes, i.e. platinum, gold or silver, do not form good interfaces for the protein electrochemical reaction. If metal electrodes do work, then the 'dilute' redox centre of the protein is usually buried within a matrix of polypeptide that creates an 'insulating' component that results in only a small and sometimes indistinguishable faradaic current from the redox centre. The more recent use of carbon-based electrodes, including glassy carbon, pyrolytic graphite and carbon nanotubes has assisted in improving this protein-electrode interface. Furthermore, non-redox active adhesives aid the attachment of the protein such that a film of protein can be interrogated, reducing the amount of protein needed as well as providing a 'soft-interface' for the protein to attach to the electrode. However, these protein-glues have been shown to modify the redox signals. In the case of a bacterial P450, P450cam, the apparent redox potential ($E^{0\prime}$) varies from −303 mV in solution to +22 mV with didodecyldimethylammonium bromide (DDAB) surfactant, measured *vs.* a standard hydrogen electrode, SHE (Fleming et al., 2006). This huge variation gives little confidence in these data as thermodynamic information and especially in terms of the physiological relevance.

Despite all these challenges, comparative studies using identical methods to immobilise the protein have proven valuable, providing both thermodynamic and kinetic information such as the redox potential $E^{0\prime}$ and the electron transfer rate constant $k^0$. We have been involved in studies using all these approaches over the past decade and provide a review of these data for P450c17. Fortunately, the electrochemical methodologies have advanced together with the expression and purification of proteins under investigation.

## 3. Introduction to electrochemical methods

The redox potential describes the ability of a chemical species to acquire electrons. Therefore it is fundamental in defining the direction in which electrons are transferred and thus determining what will be reduced and what will be oxidised. The redox potential is a relative quantity and is usually measured for an electrode reaction under standard conditions against a standard hydrogen electrode, i.e. the standard redox potential $E^0$. However, it is by definition considered to represent the reaction under equilibrium, which in practice is often not appropriate or even impossible to achieve. In fact, in voltammetric studies, where a current flows (i.e. for a reduction/oxidation reaction), the system is never truly at equilibrium. Therefore, experimentally obtained redox potentials are referred to as the formal redox potential, $E^{0\prime}$ and include additional contributions from factors, such as non-standard