Heterologous expression, purification and enzymatic activity of aldo-keto reductase 1C4

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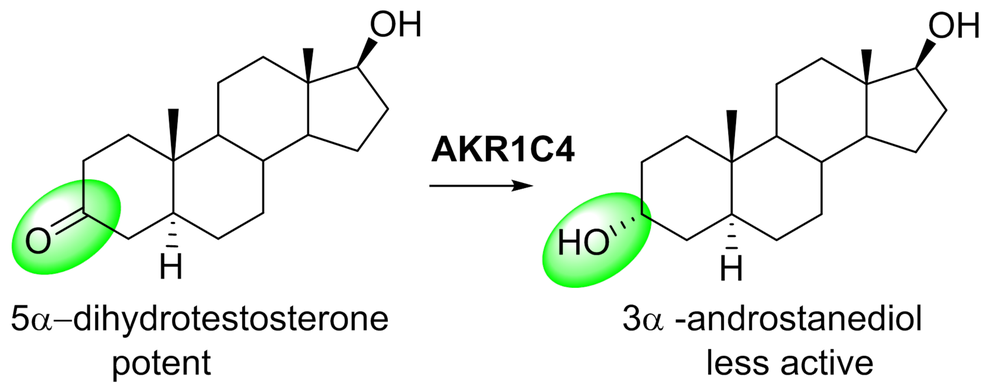
INTRODUCTION

Oxidoreductases from the aldo-keto reductase (AKR) superfamily, together with cytochrome P450 superfamily, play an essential role in the metabolism of steroids. Human AKRs are mostly monomeric cytosolic NADPH-dependent oxidoreductases involved in the metabolism of not only steroids but also a broad spectrum of other carbonyl-containing substrates, such as aldehydes and ketones, prostaglandins, retinoids, bile acids and carbohydrates. Participation of these enzymes in the detoxification of xenobiotics, carcinogen metabolites and lipid peroxidation products makes them an attractive target for research. Based on sequence similarity, there are more than one hundred members of this superfamily divided into at least 15 subfamilies. AKR1C4 is highly expressed in the liver and plays a pivotal role in xenobiotic metabolism. It preferentially acts as a 3-alpha hydroxysteroid dehydrogenase (**Figure 1**).



5α–dihydrotestosterone
potent

3α -androstanediol
less active

***Figure 1**. General reaction catalyzed by AKR1C4.*

AIM

The aim of this study was to express human AKR1C4 in *E. coli*, optimize a protein purification protocol and develop an enzyme activity assay using a known AKR1C substrate, 9,10-phenanthrenequinone (PQ), and inhibitor, ibuprofen.

MATERIAL AND METHODS

The pET28b(+)-AKR1C4 construct was a kind gift from dr Chris Bunce from The University of Birmingham[1]. Chemically competent *E. coli* BL21 cells were transformed using a heat shock procedure (**Figure 2**) and expression of protein was induced by addition of IPTG at 25°C. His-tagged AKR1C4 was purified using immobilized metal affinity and size-exclusion chromatography. Enzyme activity was monitored as the decrease in fluorescence emission at 460 nm following excitation at 340 nm. The reaction was carried out in the presence of NADPH and PQ. A known inhibitor, ibuprofen, was also tested.

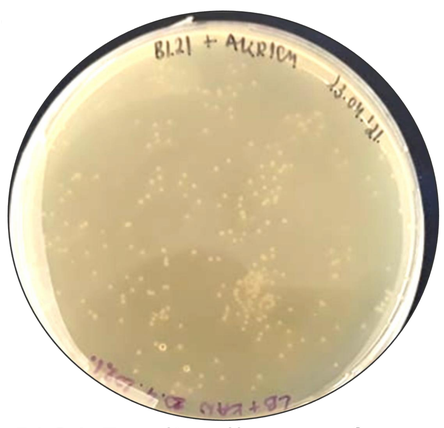


***Figure 2**. BL21 E.coli cells transformed with pET28b(+)-AKR1C4 plasmid.*

RESULTS AND DISCUSSION

His-tagged AKR1C4 was purified using immobilized metal affinity chromatography, followed by buffer exchange using a size-exclusion column. As expected, the molecular weight of recombinant AKR1C4 estimated by SDS-PAGE was ~37 kDa (**Figure 3**) and the purity of the obtained protein was sufficient for further studies. Kinetic fluorescence measurements of NADPH consumption confirmed that the enzyme was catalytically active (**Figure 4**). In the presence of AKR1C4, NADPH-dependent reduction of PQ resulted in a decrease in fluorescence over time, while no fluorescence change was observed in control reactions in the absence of enzyme. On the other hand, the slope of the curve in the presence of ibuprofen was lower vs. reaction control, suggesting enzyme inhibition by ibuprofen.

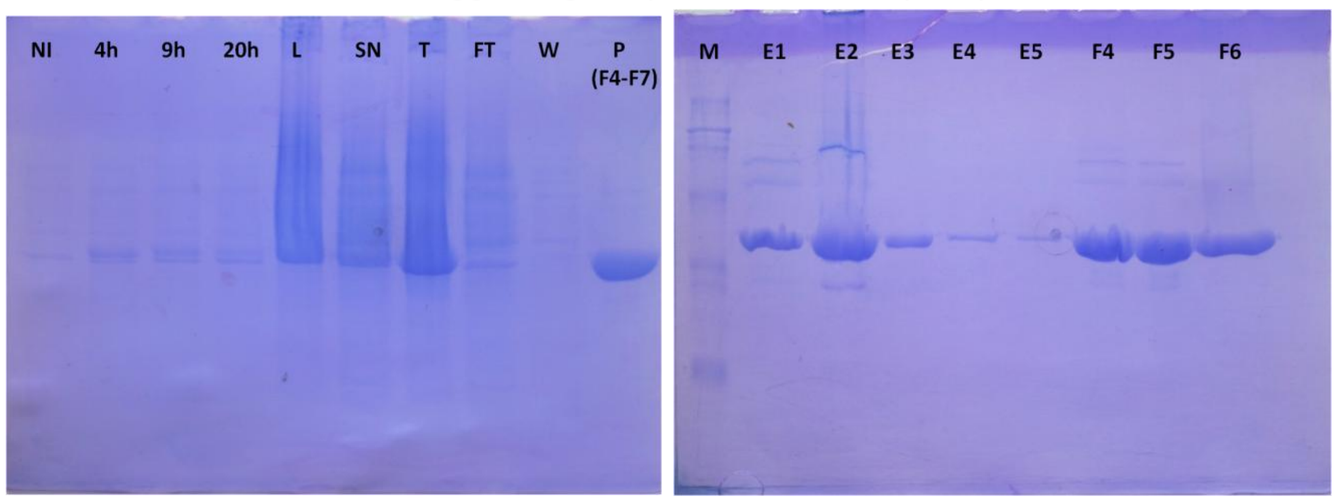


***Figure 3.** Coomassie Blue-stained SDS-PAGE gel of expression and purification steps of recombinant human His-tagged AKR1C4 in E. coli. NI- cells in which protein expression was not induced; 4h, 9h, 20h- level of protein expression 4, 9 or 20 hours after IPTG induction; L-lysate; SN-soluble fraction; T-insoluble fraction; FT-flowthrough fraction; W-fraction after washing Ni-column; P-pooled F4 and F5 fractions; M-marker; E-eluted fractions from the Ni-column; F-eluted fractions from the size-exclusion chromatography column*

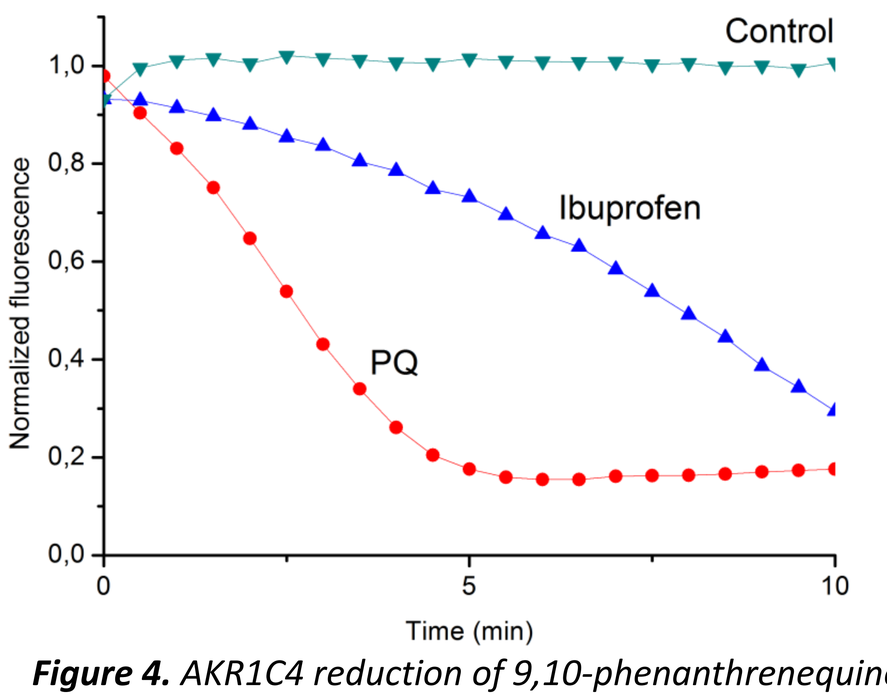


***Figure 4.** AKR1C4 reduction of 9,10-phenanthrenequinone in vitro in the absence of inhibitor (PQ) and inhibition by ibuprofen. Control-change in NADPH fluorescence intensity during time in the absence of enzyme.*

CONCLUSION

We have optimized here a protocol for purification and measurement of the enzyme activity of recombinant AKR1C4. Since imbalanced expression of human AKRs is associated with the development and progression of various diseases, such as hepatic cancer, this assay may be used for screening and identification of AKR1C4 inhibitors and design of potential therapeutics.

REFERENCES

[1]Veliça, P., Davies, N. J., Rocha, P. P., Schrewe, H., Ride, J. P., & Bunce, C. M. (2009). Lack of functional and expression homology between human and mouse aldo-keto reductase 1C enzymes: implications for modelling human cancers. *Molecular cancer*, *8*(1), 1-12.

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