Redox and Spectroscopic Properties of Human Indoleamine 2,3-Dioxygenase: Implications for Biological Catalysis

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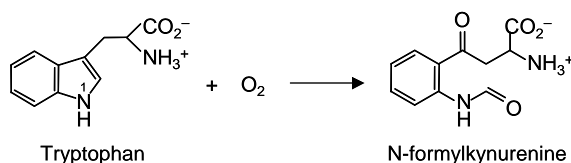
The L-kynurenine pathway – which, ultimately, leads to the formation of NAD – is the major catabolic route of L-tryptophan in mammals. The initial, rate-limiting step in this pathway is the oxidative cleavage of L-tryptophan to give N-formylkynurenine, and is catalysed by indoleamine 2,3 dioxygenase (IDO). There is a wealth of evidence linking IDO induction and kynurenine pathway metabolites to various physiological and pathophysiological conditions, including antimicrobial, antiviral and antitumour activity, and various neurological disorders.



The catalytic mechanism involves reduction of the ferric heme, binding of O_2 to the ferrous heme and binding of L-Trp, although the precise sequence – O_2 binding followed by L-Trp binding or *vice versa* – is not known. In fact, there is much that we do not understand about this important mammalian target and there are a number of questions that need urgent attention. To begin to address these deficiencies, we have developed a bacterial expression system for human IDO that allows us to isolate purified enzyme in quantities that are sufficient for functional studies. In this work, we present the redox properties of the human IDO enzyme, including the influence of substrate on the redox chemistry, and a detailed examination of the spectroscopic properties of the substrate-bound and substrate-free enzyme. We also present functional data for the H303A variant and an assessment of the likely role of this residue on substrate and dioxygen binding. The results can be used to build a more detailed picture of the possible *modus operandi* of the IDO enzyme.