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Crystallin–gazing: unveiling enzymatic activity

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μ-Crystallin is an NADPH-dependent thyroid hormone binding protein (Vie *et al*. 1997), originally discovered in the ocular lens of marsupials and subsequently found in other tissues including brain, cochlea, kidney, heart, skeletal muscle and other tissues. Its crystal structure has been solved (Cheng *et al*. 2007). Mutations in μ-crystallin, including one that inhibits binding of thyroid hormone, cause non-syndromic deafness in man (Abe *et al*. 2003; Oshima *et al*. 2006). Altered expression of μ-crystallin also occurs in other human diseases, such as Alzheimer's disease, hyperglycemia, heart failure, facioscapulohumeral muscular dystrophy and schizophrenia (Reed *et al*. 2007; Sommer *et al*. 2010; Martins-de-Souza *et al*. 2011), as well as in murine mutants of superoxide dismutase 1 (Fukada *et al*. 2007), studied as models of amyotrophic lateral sclerosis. μ-Crystallin is thought to bind to thyroid hormone in the cytoplasm of cells (Suzuki *et al*. 2007) and may deliver the hormone to the nucleus (Mori *et al*. 2002), where it plays an important role in regulating gene expression. Until the current report by Hallen *et al*. (2011), no enzymatic function for μ-crystallin has been reported.

In this study, Hallen *et al*. (2011) show for the first time that l-crystallin has ketimine reductase activity. They use mass spectroscopy to demonstrate that μ-crystallin is essentially identical to ketimine reductase purified from lamb forebrain, and show further that a purified, recombinant form of human μ-crystallin has a specific activity and pH optimum in ketimine reductase assays similar to the purified mammalian enzyme. Like the latter, it can catalyze the conversion of several different cyclic ketimines to their reduced products. Remarkably, the authors report that the most common form of thyroid hormone, 3,5,3′-triiodothyronine (T3), strongly inhibits catalytic activity at sub-micromolar concentrations.

The authors point out that the purified μ-crystallin accounts for only 0.19% of the total enzymatic activity measured in the crude brain extract. No other fractions obtained during the purification were identified as being enriched in ketimine reductase activity. This raises the possibility, acknowledged in the paper, that there may be other proteins with ketimine reductase activity in mammalian brain tissue. Alternatively, the enzyme may be unstable or subject to post-translational modifications that can alter its activity and yield. If the enzymatic function of μ-crystallin is indeed associated with any or all of the diseases mentioned above, it will be important to understand the relationships linking stability and

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catalytic activity to binding of substrates and key ligands, including NADH, NADPH and T3.

The report also raises many questions about the roles of μ-crystallin as an enzyme and as a thyroid hormone binding protein. Do the known variants of μ-crystallin have similar enzymatic activities? Does μ-crystallin have a similar affinity for NADH and NADPH *in vitro*, but as a primarily cytoplasmic enzyme does it utilize NADPH preferentially *in situ*? What are its affinities for the different forms of thyroid hormone that are biologically active, and do all of them inhibit catalytic activity as effectively as T3? What are its preferences for substrates in different tissues, and does its catalytic activity in some way regulate signaling and gene expression by thyroid hormones? If there are indeed other forms of ketimine reductase in mammalian tissues, is μ-crystallin's major function enzymatic, or, as suggested by the point mutation that inhibits T3 binding and causes deafness (Oshima *et al.* 2006), is its more likely role, at least in some tissues, to bind thyroid hormone and thereby regulate its effects on transcription? Future studies that couple assays of enzymatic activity to appropriate cellular and molecular manipulations could address these questions.

The dependence of the binding and catalytic activities of μ-crystallin on metabolism are also likely to be significant. To take only one possibility, many of the diseases mentioned above are associated with hypoxia, which typically causes acidosis. Hallen *et al.* (2011) show that μ-crystallin's enzymatic activity is optimal at acidic pH, suggesting that mild acidification of the cytoplasm in hypoxic tissues may increase its ketimine reductase activity, at least in the absence of significant levels of T3. The neuroprotective role of some of its substrates may be modulated *in vivo* by this metabolic up-regulation of activity. As the authors note, 'T3 control of levels of ketimines, which are considered putative neurotransmitters/neuromodulators, adds a new layer to the complexity of neurochemistry, suggesting subtle interactions among the endocrine system, amino acid metabolism and neurotransmission'. The current report is an important contribution to our understanding of these 'subtle interactions'.

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