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Poster presentation

NO-sensitive guanylyl cyclase: binding and dissociation of nitric oxide

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The receptor for nitric oxide (NO), NO-sensitive guanylyl cyclase, plays a key role in diverse physiological processes like smooth muscle relaxation, inhibition of platelet aggregation and synaptic plasticity. NO-sensitive guanylyl cyclase contains a prosthetic heme group serving as NO-binding site and mediating the several hundred-fold activation of the enzyme by NO.

Binding of NO to the heme group results in color changes thereby allowing direct monitoring of ligand binding to the receptor. In the absence of NO, the heme group displays an absorbance maximum of 431 nm indicative of a five-coordinated heme iron with a histidine as axial ligand. Binding of NO shifts the absorbance to 399 nm which has been attributed to rupture of the histidine to iron bond resulting in a five coordinated nitrosyl-heme. These spectral changes induced by ligand binding can be recorded by UV/vis spectroscopy.

In previous studies on NO binding using UV/vis spectroscopy, the enzyme was handled as a pure NO-binding protein, i.e. in the absence of its substrate. By monitoring binding of NO and activation of the enzyme simultaneously, we were able to demonstrate that binding of NO in the absence of the substrate MgGTP did not induce activation of the enzyme but produced an inactive conformation indistinguishable from the active enzyme by spectroscopy. Activation of this already NO-bound species required the presence of MgGTP and additional free NO at the same time. Furthermore, the substrate MgGTP or the reaction products (Mg + cGMP + pyrophosphate) altered the NO-binding process itself as they accelerated the rupture of the histidine to iron bond.

Deactivation of the enzyme is triggered by dissociation of NO from the prosthetic heme. NO dissociates from the heme of GC faster than in many other hemoproteins with half life times ranging from seconds to minutes. NO sensitizers (lead compound YC-1) have been reported to inhibit deactivation of the enzyme. By using stopped flow UV/vis spectroscopy, we analyzed whether this inhibition of deactivation was caused by either stabilization of the active conformation after NO dissociation or by inhibition of NO dissociation itself.