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Poster presentation

Formation of quasi-covalent sGC α_1/β_1-heterodimers by ODQ-induced oxidation of the prosthetic heme moiety

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Soluble guanylate cyclase (sGC), a heme containing α/β-heterodimer, is one of the crucial enzymes within the NO/cGMP signaling pathway. The enzyme becomes activated up to 200-fold upon binding of its physiological activator nitric oxide (NO) to the prosthetic heme moiety. sGC activation by NO requires the presence of the reduced Fe^{2+} heme moiety and oxidation to its ferric Fe^{3+} state abolishes any NO-induced enzyme activation. Based on this observation specific sGC inhibitors such as ODQ were developed which show a higher specificity for the sGC heme than general heme oxidants such as methyleneblue or ferricyanide.

In the present work we report an unexpected side effect of the sGC inhibitor ODQ. Incubation of sGC expressing cells with ODQ resulted in the formation of a sGC α_1/β_1 immunoreactive protein of a molecular mass of about 160 kDa. This protein, herein after referred to as p160, was identified by mass spectrometry as sGC α_1/β_1 heterodimer. Further studies revealed a non-covalent coupling of both subunits. The formation of p160 requires the presence of the heme moiety as proved by the expression of the constitutive heme-deficient sGC mutant $\beta H^{105}F$. These results were supported by findings that the NO-independent sGC activator BAY 58-2667, which was shown to activate sGC by replacing the weakly bound oxidized heme also prevents the formation of p160 [1,2]. Therefore it can be hypothesized that the sGC heme moiety catalyzes an up to now unknown ODQ-based reaction by which the sGC heme becomes oxidized and the heterodimer is converted into the quasi-covalent coupled p160 probably by chemical modification of various residues. BAY 58-2667 protected sGC from this ODQ-mediated conversion and, in addition, appeared to stabilize the sGC β_1-subunit resulting in an increased protein level of this subunit.

In summary, we were able to show the formation of a previously unrecognized state of sGC, the quasi-covalent coupled α_1/β_1 heterodimer p160, which formation is initiated by ODQ and is dependent on the presence of the sGC heme moiety. Furthermore, we could show that the heme-mimic BAY 58-2667 diminished the formation of p160 and stabilizes the sGC β-subunit resulting in increased protein levels of this subunit. However, whether p160 plays a physiological role remains unclear and will be investigated by further studies.

References

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