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NO-sensitive guanylyl cyclase: Identification and purification of the dimerization domain

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The nitric oxide (NO)-sensitive guanylyl cyclase plays a key role in diverse signalling pathways by catalysing the biosynthesis of the messenger molecule cGMP. To date, two isoforms of the enzyme ($\alpha_1\beta_1$ and $\alpha_2\beta_1$) are known, both of which contain a prosthetic heme group bound to the histidine 105 of the β_1 subunit. The $\alpha_1\beta_1$ isoform is ubiquitously expressed and is considered to be soluble, whereas the $\alpha_2\beta_1$ isoform is mainly expressed in brain and is located to the membrane via interaction with PSD-95. The three-dimensional structure of NO-sensitive guanylyl cyclase has not been solved. Yet, by sequence comparison the subunits are generally divided into three domains. An N-terminal regulatory domain, a central domain postulated to be involved in dimerization and a C-terminal catalytic domain. For catalytic activity dimerization of the α and β subunit is mandatory, but until recently the regions involved in the interaction were unknown.

By coprecipitation of several deletion mutants of the α_1 and β_1 subunit, expressed in Sf9-cells, we have mapped the α_1 dimerization domain to amino acids 61-462. Within this region, we identified two binding sites. One, as postulated, covering the central amino acids 367-462, the other, quite unexpectedly, located in the N terminus of the α_1-subunit (amino acids 61-128). By itself neither region was sufficient to mediate full dimerization. Of the β_1 subunit the N-terminal and central amino acids 1-385 exhibited wt-like binding to the identified α_1 domain. To further characterize the short, but stable, dimeric NO-sensitive guanylyl cyclase we purified the mutant from Sf9-cells and analysed heme-coordination and binding of NO in spectrophotometric measurements. The dimeric state of the mutant was confirmed by gelfiltration analysis.

As β_1 is the binding partner for both the α_1 and α_2 subunit, we reasoned that dimerization of the $\alpha_2\beta_1$ isoform must be mediated by parts of the α_2 subunit homologous to the α_1 dimerization domain. Accordingly, we cloned the respective sequences of α_2 (amino acids 76-501) and analysed binding of the mutant to the N-terminal and central regions of β_1.