Previously characterized mammalian soluble guanylyl cyclases form α / β heterodimers that can be activated by the gaseous messenger, nitric oxide and the novel guanylyl cyclase modulator YC-1. Four mammalian subunits have been cloned, named α_1, β_1, α_2 and β_2. The α_1 / β_1 and α_2 / β_1 heterodimeric enzyme isoforms have been rigorously characterized. The role of the β_2 subunit has remained elusive. Here we isolate a novel variant of this subunit and show that the β_2 subunit does not need to form heterodimers for catalytic activity because enzyme acivity can be measured when it is expressed alone in Sf9 cells. In analogy to the β_3 subunit recently isolated from the insect Manduca sexta, activity was dependent on the presence of 4 mM free Mn^{2+}. The EC_{50} values for the NO-donor DEA/NO were shifted to the left by one order of magnitude as compared with the α_1 / β_1 heterodimeric form. In the presence of the detergent tween, NO-sensitivity of β_2 was abolished but the enzyme could be activated by protoporphyrin IX, indicating removal of a prosthetic heme group and exchange for the heme precursor. We suggest that the β_2 subunit is the first mammalian NO-sensitive guanylyl cyclase lacking a heterodimeric structure.

Nitric oxide-sensitive guanylyl cyclase is a heterodimeric enzyme consisting of one α and one β subunit. Here, we clone the first α_2 subunit ortholog and functionally express the cDNA in Sf9 cells. Our data indicate a high degree of conservation of the primary sequence and functional activity of the rat α_2 subunit. Soluble guanylyl cyclase (sGC) is the only conclusively proven receptor for nitric oxide (NO). The heterodimeric enzyme consists of one α and one β subunit and catalyzes the reaction of GTP to the second messenger cGMP. The α_1 and β_1 subunits were first purified as a heterodimer from bovine and rat lung and were subsequently cloned. Co-expression of the cloned subunits (α_1 and β_1) yielded NO-sensitive guanylyl cyclase activity. Two other subunits have been identified by use of the polymerase chain reaction or low stringency hybridization from human fetal brain (α_2) and rat kidney

(β_2). Co-expression experiments using the novel cDNAs yielded NO-sensitive guanylyl cyclase activity for the α_2 / β_1 combination. The α_2 subunit has been detected on the protein level in human placental cytosol after precipitation with a β_1 subunit antibody. Here we identify the first full-length ortholog sequence of the α_2 subunit of soluble guanylyl cyclase and functionally express an enzyme containing the newly cloned subunit in Sf9 cells. Co-expression of the rat α_2 subunit with the β_1 subunit in Sf9-cells yielded an NO-sensitive enzyme. The degree of sequence conservation between human and rat was slightly higher for α_2 (90 %) than α_1 (89 %). The N-terminal third is highly conserved between the α_2 orthologs (87 %) but shows only little sequence homology between the α_1 and α_2 subunits (27 %). Deletion of the first 131 amino acids of the α_1 subunit resulted in an enzyme that was NO-insensitive due to a significantly reduced capacity to bind heme. Zhao and Marletta (1997) have presented data indicating that the N-terminal part of the β_1 subunit is sufficient for heme binding. Deletion mutants of the α_2 subunit were characterized with respect to sensitivity towards NO and YC-1 and kinetic parameters in the Sf9-system.