

## Structure activity relationships and analog design using the single chain model

Irving Boime, Vicenta Garcia-Campayo, and Albina Jablonka-Shariff  
Department of Molecular Biology & Pharmacology, Washington University School of Medicine, St. Louis, MO, USA

One of the major developments in exploring structure-activity relationships of the glycoprotein hormone family was the genetic engineering of single chains comprised of the common  $\alpha$  subunit and one or more of the hormone-specific  $\beta$  subunits tandemly arranged. Use of single chain chimeras containing modifications in the  $\alpha$  or  $\beta$  subunits that otherwise inhibit heterodimer formation results in biologically active analogs. Thus, inter-subunit interactions are essential for the intracellular trafficking of the heterodimers but are permissive for receptor binding and signaling. That the cognate receptors recognize the  $\alpha/\beta$  domains in different conformations implies extensive flexibility between the ligand and the receptor. To examine this issue further, a triple-domain gene chimera encoding the sequence FSH $\beta$ -CG $\beta$ - $\alpha$  was engineered. This analog, composed of a single  $\alpha$  subunit covalently linked with two tandemly arranged  $\beta$  subunits, displayed high-affinity binding to the corresponding human receptors. Although this chimera exhibited dual gonadotropin activity, it was unclear if a single molecule possessed both FSH and CG activities or if two species were generated, each corresponding to a single activity. To distinguish between these possibilities, a preadsorption receptor binding protocol was used in which medium containing the secreted form of the analog was preincubated with CHO cells expressing either the LH/CG or FSH receptors followed by binding to cells expressing either receptor. If a single molecule exhibited bifunctional activity, regardless of which receptor cell line is used in the initial preincubation, both of the activities in the secondary binding assays would be reduced in parallel. Alternatively, if there were two distinct biologically active species, there would be a preferential loss of a single activity following the first incubation. The results are consistent with the hypothesis that at least two distinct bioactive populations of chimera are secreted, each corresponding to a single activity. However, it cannot be excluded that only one molecule demonstrating dual activity is also formed. Because these bulky single-chain variants are bioactive compared to the native heterodimer, this demonstrates flexibility in the ligand-receptor interaction. The recent crystallographic studies, demonstrating that FSH undergoes extensive conformational changes when binding to the extracellular domain of its receptor, support all of the above observations. While both FSH and LH are synthesized in the gonadotroph, it is unclear how in the endoplasmic reticulum the  $\beta$  subunits sort from each other and assemble with the  $\alpha$  subunit. Our data provide an explanation for this *in vivo* segregation of the corresponding heterodimers in the same cell: Once the  $\alpha$ - $\beta$  contacts are formed, the resultant species are stable.