Determination of Three-Dimensional Structures of the Thyroid Hormone Receptor Complexes using Crosslinking Constraints and Bioinformatics

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Introduction

Thyroid gland is one of the largest endocrine glands in the human body, which secretes two thyroid hormones: thyroxine (3,5,3',5'-tetraiodo-L-thyronine, known as T4) and triiodothyronine (3,5,3'-triiodo-L-thyronine, known as T3). The T3 hormone act in complex and specialized systems through the interaction with its receptor, thyroid hormone receptor (TR), and regulates the transcription of specific human genes.¹

There are several pathologies associated with disorders caused by thyroid hormones, including the Grave's disease, Hashimoto's syndrome, cretinism, hyperthyroidism, and hypothyroidism;¹ so understanding how the TR interact with T3, with other nuclear receptors (for example, Retinoid X Receptor – RXR), and coregulators (as Glucocorticoid Receptor-Interacting Protein 1 - GRIP1) is relevant to combat these disorders. Therefore, structural dynamics of TRβ-RXRα-GST_GRIP1_LBD complexes, with and without ligands, were analysed via chemical crosslinking method using disuccinimidyl suberate (DSS) and “bottom-up approach” strategy² by mass spectrometry. The distance constraints are been used in bioinformatics to determinate the three-dimensional structures of these protein complexes.

Results and Discussion

Spectra of fragment ions of the intra- and intermolecular lysine and serine cross-linked peptides (Lys-Lys, Ser-Ser, and Lys-Ser) were identified by Mascot Server and SIM-XL program. These data revealed the presence of interactions in different regions of TRβ-RXRα-GST_GRIP1_LBD complexes, with and without the 9-cis-retinoic acid. Based on these distance constraints of the intramolecular cross-linked peptides was possible to obtain five molecular models for each individual protein of the complexes by PyMOL, I-TASSER, and Xwalk software, suggesting a likely structure for each protein due to constraints (Fig. 1).

Conclusions

These results demonstrate a conformational dynamic of the protein complexes and help to understand the system of TR activation. Now, these data will be evaluated by Rosetta modeling software for a more precise structural prediction of these macromolecular complexes.

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Figure 1. Protein structures are shown in green color in ribbon representation and atoms of disuccinimidyl suberate are shown as colored spheres. (A) Absence and (B) presence of 9-cis-retinoic acid in the TRβ-RXRα-GST_GRIP1_LBD complexes.

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