

## アンドロゲン受容体蛍光トレーサーの設計合成と受容体結合特性

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環境化学物質の受容体結合試験において、非放射性的の蛍光リガンドはその簡便さと経済性より非常に有用である。本研究では、アンドロゲンの蛍光リガンドを用いた受容体結合試験系の確立を目的として、アンドロゲンの1種ジヒドロテストステロンに、蛍光核フルオレセインを一連のアルキルリンカーで架橋した誘導体を設計・合成した。化学合成は、ジヒドロテストステロンへのアルキル架橋鎖の導入、次いでフルオレセインの導入の2段階、または先にフルオレセインへのアルキル架橋鎖の導入、次いでジヒドロテストステロンの導入の2段階で行い、ゲルろ過、HPLCにより精製した。合成した化合物について<sup>[3H]</sup>ジヒドロテストステロンをトレーサーとした競争結合試験を実施し、アンドロゲン受容体への結合親和性を評価した。

### Design and synthesis of fluorescent tracers for the fluorometric androgen receptor binding assay

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For rapid screening of chemicals which might have the potential to interact with the androgen receptor (AR), the fluorescent ligand is crucially important. This is because the assay conditions should be very mild for the receptor conformation. In the present study, 5 $\alpha$ -dihydrotestosterone (DHT) was conjugated with fluorescein through alkyl linkers of various methylene cross-linking lengths. DHT was first converted into 17 $\beta$ -hemisuccinate with succinic anhydride/4-dimethylaminopyridine.  $\omega$ -Cbz-polymethylenediamines were then coupled with 5-(and-6)-carboxyfluorescein succinimidyl ester to afford  $\omega$ -Cbz-polymethylenediamine-carboxyfluorescein. After removal of the Cbz group by TFA, the resulting 17 $\beta$ -DHT hemisuccinate was reacted with hydroxysuccinimide to afford its succinimidyl ester. Obtained  $\omega$ -polymethylenediamine-carboxyfluorescein was further reacted with this succinimidyl ester to give the final product fluorescein-conjugated DHT. Each compound synthesized was purified by gel filtration followed by reversed-phase HPLC. Purified fluorescent ligands were examined in the binding assay for AR using [<sup>3</sup>H] DHT as a tracer. All these synthetic data and receptor binding data will be discussed to find a better fluorescent tracer for receptor binding assay.