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Author	高梨, 浩典(Takanashi, Hironori) 本間, 浩(Honma, Hiroshi) 松井, 道夫(Matsui, Michio)
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Separation and Purification of Uridine Diphosphate-Glucuronosyltransferases by a Chromatofocusing on a High-Performance Liquid Chromatograph*

Hironori TAKANASHI, Hiroshi HOMMA and Michio MATSUI

高梨浩典，本間　浩，松井道夫

A rapid method for the separation and purification of uridine diphosphate-glucuronosyltransferases (GT) was developed with the use of chromatofocusing on high-performance liquid chromatograph. GT isoenzymes solubilized from hepatic microsomes of Wistar rats were separated on a Mono P column, a pre-packed column for chromatofocusing. Using 4-nitrophenol, testosterone and androsterone as substrates, four fractions with different GT activities were separated in a pH gradient from 9.5 to 7.0. Two isoenzymes, testosterone GT and androsterone GT were purified to apparent homogeneity. They were eluted at pH 8.9 and 8.0 and had subunit molecular weight values of 50000 and 52000, respectively. Approximately 10 mg of solubilized microsomal proteins were applied and the elution was completed within 2 hr. Addition of N-nitrodiethylamine, an *in vitro* activator of GT activity, enhanced the GT activity toward 4-nitrophenol in the three fractions. This chromatographic analysis confirmed the absence of androsterone GT isoenzyme in LA Wister rats, a mutant strain in terms of androsterone glucuronidation.

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