Subject: Separation of type S1 Lipoprotein (a) by ultracentrifuge

Model: High performance preparative ultracentrifuge CP100 α

Rapid (for 4.5 hours) and high purified separation of type S1 lipoprotein (a) using fixed-angle rotor P100AT2.

Human serum lipoprotein is thought to have something to do with atherosclerosis or hyperlipidemia and recently the association is investigated in various ways. Lipoprotein (a) (Lp (a)) is recently investigated the association with atherosclerosis\(^1\) after the report that the concentration in serum is a major risk factor for heart diseases has been published\(^3\). But the density of Lp (a) is close to the LDL and HDL. Thus it has been difficult to separate pure Lp (a) by density gradient ultracentrifugation. Then we recognized the difference of the molecular size between Lp (a) and other lipoprotein and tried to separate Lp (a) using rate zonal separation method. This method is based on the character that large size of lipoprotein floats faster than that of small one. Here we tried to separate Lp (a) especially type S1 Lp (a) that was thought to have much to do with atherosclerosis or hyperlipidemia than the other Lp (a)\(^4\).

1. Models used
   - Centrifuge: High performance preparative ultracentrifuge CP100 α
   - Rotor: P100AT2 (Fixed-angle rotor)
   - Tube: 4.7 PC thick-walled tube (Single use only)
   - Cap: B-Ti lid (Be sure to use)

2. Result
   (1) Polyacrylamide-gel-electrophoresis* (Fig. 1)
   (2) Recovery of Lp (a) and HDL (Table 1)

* Reagend kit for polyacrylamide gel lipoprotein disk electrophoresis: Lipofor (Joco Co., Ltd. Tokyo, Japan)
** Tyntriaise Lp (a) (Cosmo-Bio Co., Ltd. U.S.A.)
***Automatic analyzer: Pramax plus (Baxter Co., Ltd.)
3. Sample preparation

1.5ml of human serum
+0.5ml of soln.A + 1ml of soln.B (mix well gently)

100,000rpm, 3h, 16°C
Acc.:”9”, Dec.”7”.

1ml of top layer (VLDL, LDL)
0.5ml of lower layer (HDL)

1.5ml of middle layer
+0.15ml of soln.C (mix well gently)
(include Lp (a)) ............... soln.1

Layer 2ml of soln.D on 1ml of soln.1
in 4.7 PC thick-walled tube

100,000rpm, 1.5h, 16°C
Acc.”5”, Dec.”7”.

1ml of top layer: Purified Lp (a).

Soln. A (1.006g/cm³) : Dissolve 11.4g of NaCl and 0.1g of EDTA-Na₂ with 500ml of distilled water (DW) and add 1ml of 1N NaOH. Then add DW up to 1000ml and add 3ml of DW. (NaCl : 0.195mol)

Soln. B (1.182g/cm³) : Dissolve 24.98g of NaBr to 100ml of soln.A. (NaCl : 0.195mol, NaBr : 2.44mol)

Soln. C (1.478g/cm³) : Dissolve 78.32g of NaBr to 100ml of soln.A. (NaCl : 0.195mol, NaBr : 7.65mol)

Soln. D (1.080g/cm³) : Mix soln. A with soln. B at the volume ratio of 1.3 : 1.0.

Reference