HITACHI

himac APPLICATION

No. 70 April 1997

Subject Separation of type S1 Lipoprotein (a) by ultracentrifuge

Model High performance praparative ultracentrifuge CP100α

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, 2] 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 lipoproteis 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 CP100α
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)

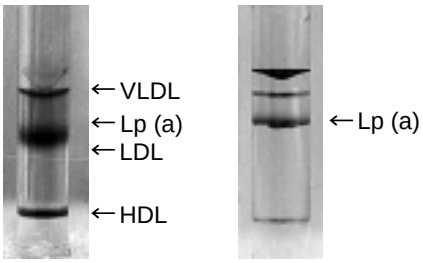


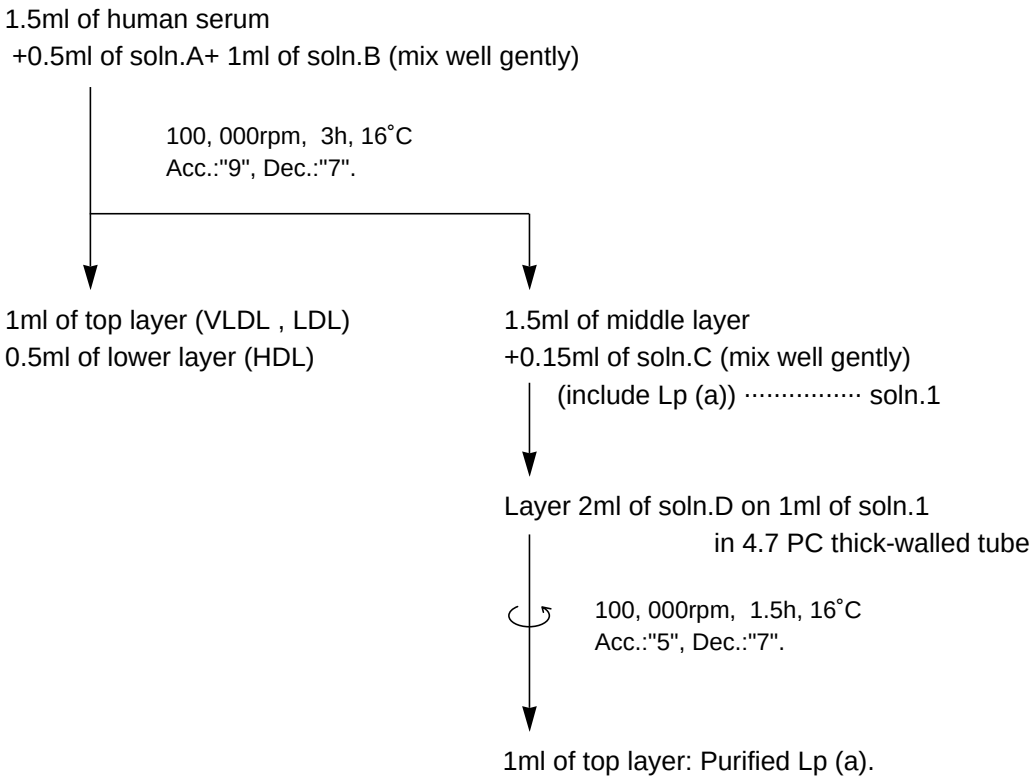
Fig. 1

Table 1

	La (a)** (mg/ml)	HDL - cholestrol*** (mg/ml)
Original serum	72.5	78.5
Separated Lp (a)	17.5 (Recovery : 21.8%)	Not detected

* Reagend kit for polyacrylamide gel lipoprotein disk electrophresis : Lipofor (Joco Co., Ltd. Tokyo, Japan)
** Tyntriase Lp (a) (Cosmo-Bio Co., Ltd. U.S.A.)
***Automatic analizer : Pramax plus (Baxter Co., Ltd.)

3. Sample preparation



Soln. A (1.006g/cm^3) : 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/cm^3) : Dissolve 24.98g of NaBr to 100ml of soln.A. (NaCl : 0.195mol, NaBr : 2.44mol)

Soln. C (1.478g/cm^3) : Dissolve 78.32g of NaBr to 100ml of soln.A. (NaCl : 0.195mol, NaBr : 7.65mol)

Soln. D (1.080g/cm^3) : Mix soln. A with soln. B at the volume ratio of 1.3 : 1.0.

Reference

1) Utermann G., *Science*, 246 , 904 (1989).

2) Fless G.M., *J.Biol.Chem.*, 267 , 339 (1992).

3) Metcalfe J.C., *Nature,* 370 , 460 (1994).

4) Utermann G., et al., *J. Clin.Invest.*, 80 , 458 (1987).

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