himac APPLICATION
No.131
Jan. 2008

Separation and purification of hepatitis B antigens using a zonal rotor – (2)

CP-WX series preparative ultracentrifuge/P35ZT zonal rotor

The hepatitis B antigens separated as described in himac APPLICATION No. 130 were fractionated into Dane particles (viral particles, about 42 nm in diameter), small particles and tubular particles (HBs antigens about 27 nm in diameter) by cesium chloride density-gradient equilibrium centrifugation as follows.

Experiment

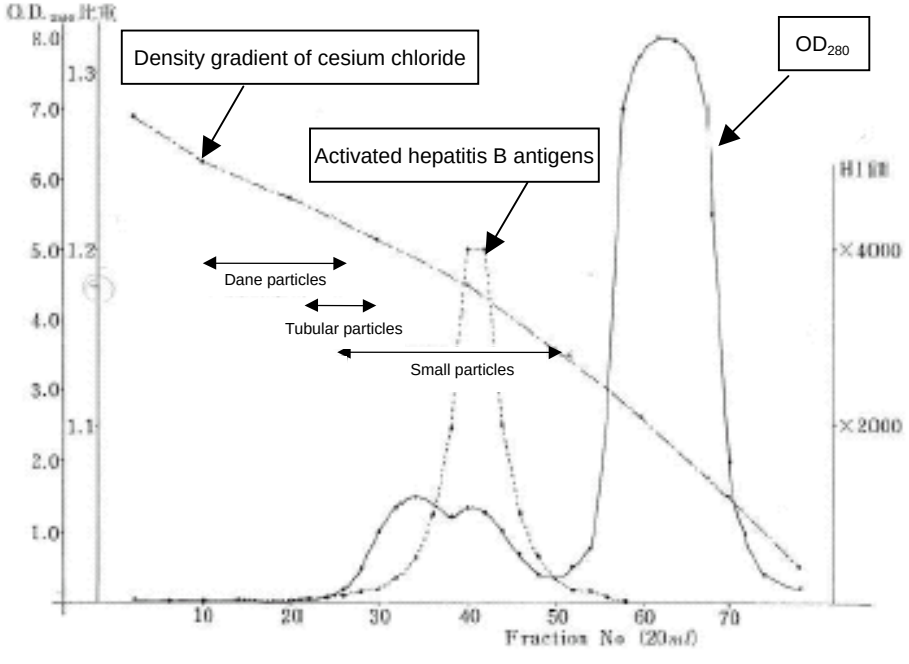
1. Conditions for centrifugation

Centrifuge: CP-WX series preparative ultracentrifuge
Rotor: P35ZT zonal rotor
Speed: 28,000 rpm
Time: 12 hours
Density gradient solution:

(1) Pour 1,600 ml of the continuous density gradient solution whose cesium chloride density is 1.10 g/ml (12% (w/w)) to 1.30 g/ml (30% (w/w)) into the outer wall side of the rotor.
(2) Add about 200 ml of solution whose cesium chloride density is 1.35 g/ml (35%(w/w)) to the outer wall side of the rotor to make the inner wall side of the rotor overflow certainly.
(3) Pour 50 ml of sample into the inner wall side of the rotor. Then add about 200 ml of saline to the inner wall side of the rotor to flush out the sample remained in the pipe into the rotor completely.

Sample: Dialyze 400 ml of hepatitis B antigen fraction prepared as described in himac APPLICATION No. 130 to remove cesium chloride. Concentrate it to 50 ml by ultrafiltration. Add 7% (w/w) cesium chloride solution so that its density becomes 1.05 g/ml.

2. Result



(1) Hepatitis B antigen components were sedimented and separated at each density position.
Dane particles: 1.23 g/ml to 1.24 g/ml
Tubular particles: 1.22 g/ml
Small particles: 1.14 g/ml to 1.20 g/ml
(2) Serum proteins were found at the position of 1.06 g/ml to 1.13 g/ml.

3. Explanation

Generally, the density of serum proteins is about 1.3 g/ml. As a result of this experiment, however, the density of serum proteins was between 1.06 g/ml and 1.13 g/ml. It means that serum proteins could not be sedimented to their primary density position under the conditions for centrifugation in this experiment due to the low sedimentation coefficient of serum proteins. While hepatitis B antigen components were separated by the sedimentation equilibrium method, serum proteins were separated by the sedimentation velocity method in this experiment. Therefore, serum proteins may get into the fraction of small particles if the centrifugation time is too long. Extra care must be taken when setting the centrifugation time and speed.

Instruments

CP-WX series preparative ultracentrifuge

P35ZT zonal rotor

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