Surface Plasmon Analysis of Phosphorylated β-Casein Using a Phos binding reagent (Phosbind) Biotin LC and Streptavidin-Bound Sensor Chip

1. Ligand binding procedures

1) A sensor chip, onto which streptavidin was bound (Sensor Chip SA, made by Biacore), was set in Biacore J (made by Biacore).
2) A 10 mM aqueous HEPES-sodium hydroxide solution (pH 7.4), containing $5X10^{-3}$%(w/v) Tween 20, 0.20 M sodium nitrate, and 10 μM zinc nitrate was used as the running buffer. The sensor chip temperature was set at 25°C and the flow rate of the running buffer was set at 30 μL/min. The running buffer was made to flow until the surface plasmon resonance value stabilized. This procedure was carried out on flow cell A.
3) As a biotin derivative of Phosbind, Phosbind Biotin LC was used and this was dissolved in the running buffer ($5X10^{-3}$%(w/v) Tween 20, 0.20 M sodium nitrate, and 10 μM zinc nitrate in 10 mM aqueous HEPES-sodium hydroxide solution (pH 7.4)).
4) Binding onto flow cell A was carried out for 6 minutes at a ligand concentration of 1.0 mM, a temperature of 25°C, and a flow rate of 30 μL/min.
5) For the interaction of the Phosbind Biotin LC and streptavidin, a surface plasmon resonance signal of 240RU was obtained for the maximum binding amount.
6) A sensor chip (Phosbind sensor chip), having a Phosbind-bound sensor, was prepared by the above procedures.

2. Surface plasmon analysis of β-casein

1) β-casein (pentaphosphorylated protein, Sigma Co., Ltd.) was used as the sample to be analyzed, and this sample was dissolved in the running buffer ($5X10^{-3}$%(w/v) Tween 20, 0.20 M sodium nitrate, and 10 μM zinc nitrate in 10 mM aqueous HEPES-sodium hydroxide solution (pH 7.4))

(sample solution).

2) The surface plasmon resonance analysis was carried out in flow cell A at a sample concentration of 1.5 μM, a temperature of 25°C, a flow rate of 30 μL/min, a binding time of 15 minutes and a dissociation time of 10 minutes.

3) After measurement, the sensor chip was reactivated (removal of residual bound substances) by the addition of a 400 mM aqueous phosphate solution (pH 7.0) for 6 minutes, a 200 mM aqueous EDTA (pH 8.0) solution for 6 minutes and the abovementioned running buffer for 5 minutes.

4) The measurement results are shown in FIG. 1.

5) The results of FIG. 1 shows that a high RU value is obtained for each flow cell and that the maximum binding amount for flow cell A is 2056.

3. Interaction of the streptavidin sensor chip and β-casein

1) β-casein (pentaphosphorylated protein, Sigma Co., Ltd.) was used as the sample to be analyzed, and this sample was dissolved in the running buffer ($5X10^{-3}$%(w/v) Tween 20, 0.20 M sodium nitrate, and 10 μM zinc nitrate in 10 mM aqueous HEPES-sodium hydroxide solution (pH 7.4)) (sample solution).

2) The surface plasmon resonance analysis was carried out in flow cell A of a new sensor chip at a sample concentration of 1.5 μM, a temperature of 25°C, a flow rate of 30 μL/min, a binding time of 15 minutes and a dissociation time of 10 minutes.

3) There was absolutely no interaction of β-casein with streptavidin.