

# Surface Plasmon Analysis of Phosphorylated $\beta$ -Casein Using a Phosbind Biotin LC (PB-B 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  $5 \times 10^{-3}\%$  (w/v) Tween 20, 0.20 M sodium nitrate, and 10  $\mu$ M 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  $\mu$ L/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 Phos binding reagent, Phosbind Biotin LC was used and this was dissolved in the running buffer ( $5 \times 10^{-3}\%$  (w/v) Tween 20, 0.20 M sodium nitrate, and 10  $\mu$ M 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  $\mu$ L/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 (Phos binding reagent sensor chip), having a Phos binding reagent-bound sensor, was prepared by the above procedures.

## 2. *Surface plasmon analysis of $\beta$ -casein*

- 1)  $\beta$ -casein (pentaphosphorylated protein, Sigma Co., Ltd.) was used as the sample to be analyzed, and this sample was dissolved in the running buffer ( $5 \times 10^{-3}\%$  (w/v) Tween 20, 0.20 M sodium nitrate, and 10  $\mu$ M 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  $\mu\text{M}$ , a temperature of 25°C, a flow rate of 30  $\mu\text{L}/\text{min}$ , 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 $\beta$ -casein***

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- 1)  $\beta$ -casein (pentaphosphorylated protein, Sigma Co., Ltd.) was used as the sample to be analyzed, and this sample was dissolved in the running buffer (5X10<sup>-3</sup>%(w/v) Tween 20, 0.20 M sodium nitrate, and 10  $\mu\text{M}$  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  $\mu\text{M}$ , a temperature of 25°C, a flow rate of 30  $\mu\text{L}/\text{min}$ , a binding time of 15 minutes and a dissociation time of 10 minutes.
- 3) There was absolutely no interaction of  $\beta$ -casein with streptavidin.