Analysis of binding affinity of sugars to concanavalin A by surface plasmon resonance sensor BIACORE

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Abstract. The observed dissociation constant k_d of the lectin concanavalin A (ConA) from glycoprotein asialofetuin (ASF) changed with the concentration of inhibitory sugars. The reciprocal of k_d showed a linear relationship with the reciprocal of sugar concentration. This regression line was found to be theoretically available in the analysis of the kinetics in the interaction of sugars with ConA under conditions where the binding constant K_i of sugars to ConA is more than about 333.

1. Introduction

We previously proposed a quasi-equilibrium equation for the interaction between sugars and concanavalin A (ConA), which has been associated with the immobilized glycoproteins such as asialofetuin (ASF) on an optical biosensor chip [3]. Equation (1) represents the theoretical correlation of sugar binding to ConA.

$$\frac{1}{k_d} = \frac{1}{k_-} + \frac{k_+(R_0 - R_t)}{k_-k_{tr}} \frac{1}{K_i[S] + 1}. \quad (1)$$

In this equation, k_d represents the observed dissociation constant of ASF-ConA complex in the presence or absence of inhibitory sugars. The kinetic constants k_+ and k_- are the intrinsic association and dissociation constants of the complex, respectively. The k_{tr} represents mass-transport rate constant of the free ConA from the stationary phase to mobile phase in the flow-cell of the biosensor chip. R_0 and R_t are biosensor responses correlated to the ConA binding amount to the immobilized ASF at time 0 and time t , respectively. Under equilibrium state, the inhibitory sugar S , the free concentration of which is shown by $[S]$, binds to ConA according to the association constant K_i . We found that k_d changes with $[S]$ according to Eq. (2).

$$\frac{1}{k_d} = \nu + \frac{1}{\kappa} \frac{1}{[S]}. \quad (2)$$

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Equation (2) holds under conditions $[S] \gg 1/K_i$. The parameters κ and ν represents the affinity of the sugar with ConA and the intrinsic association of ConA with ASF in the absence of sugars, respectively, as shown by Eqs (3) and (4).

$$\kappa = K_i k_- k_{tr} / \{k_+ (R_0 - R_t)\}, \quad (3)$$

$$\nu = 1/k_-. \quad (4)$$

All the sugars should take the same ν , being independent of the inhibitory sugars. In fact, the obtained ν values with sugars having higher κ values were the same, whereas those with the lower κ values were not, being greater than those with higher κ values. These results could be due to the fact that sugars with higher κ values bind to ConA under conditions of $[S] \gg 1/K_i$, but the binding of those with lower κ values to ConA do not satisfy the above conditions. In the present study, we examined further the binding of various sugars to ConA for understanding the theoretical background of Eqs (1)–(4).

2. Experimental

Chemicals and reagents were of the highest grade commercially available. The interaction of sugars to ConA was determined with an optical biosensor as described previously [3].

3. Results and discussion

The effects of D-glucose (Glc), D-glucosamine (GlcNH₂), *N*-acetyl-D-glucosamine (GlcNAc), D-mannose (Man), D-mannosamine (ManNH₂), and *N*-acetyl-D-mannosamine (ManNAc) on the dissociation of the ASF–ConA complex were examined. As shown in Fig. 1, Gal showed no appreciable effect on the ASF–ConA complex. On the contrary, other sugars increased the dissociation of the ASF–ConA complex in a dose-dependent manner, the effect of Man being the highest. Then, we analyzed these effects according to Eq. (2), as shown in Fig. 2, and the parameters ν and κ are summarized in Table 1. We found that values of ν and κ for Man, Glc, and GlcNAc were agreed with the values reported previously [1–3].

Fig. 1. Effects of sugars on the dissociation of the ASF–ConA complex. The effects of Man (closed circles), ManNH₂ (closed triangles), ManNAc (closed rectangles), Glc (open circles), GlcNH₂ (open triangles), and GlcNAc (open rectangles) on the dissociation of ConA from ASF were examined.

Fig. 2. Double reciprocal plots for the effects of inhibitory sugars on the ConA-ASF complex. The results shown in Fig. 1 were plotted according to Eq. (2). Sugars are as for Fig. 1.

Table 1

Experimental parameters ν and κ determined for various sugars according to Eq. (2)

Sugar	Intercept (ν) ^a	Slope ^a	κ ^b	r^2 ^c
Man	139.0	19.37	0.0516	1.000
Glc	197.1	63.69	0.0157	0.999
GlcNH ₂	219.6	58.68	0.0170	0.998
GlcNAc	129.6	56.94	0.0176	0.989
ManNH ₂	338.0	90.34	0.0111	0.999
ManNAc	340.6	95.65	0.0105	0.997

^a The intercept and slope were obtained by linear regression analyses of the $1/[S]$ and $1/k_d$ plots.

^b The reciprocal of the slope according to Eq. (2).

^c Square of correlation coefficient.

In Table 1, the obtained ν values were not the same, being increased with κ values. This could be due to the error caused by the fact that the logistic term of the theoretical Eq. (1) is not taken into consideration in Eq. (2). This error seems significant with higher κ values. To know the limitation of Eq. (2), we examined how the parameter ν changes with κ for various K_i . We simulated the theoretical curves according to Eq. (1) for K_i values of 33 (closed squares), 100 (open squares), 333 (close triangles), 1000 (open circle), and 3333 (close circles) by fixing the value of $k_-k_{tr}/\{k_+(R_0 - R_t)\} = 3 \times 10^4$, and $1/k_- = 100$. The results are shown in Fig. 3, and the K_i -dependent changes of ν are shown in Fig. 4.

It was found that the ν values were almost the same in the region of $K_i > 330$. Therefore, it is concluded that Eq. (2) is available for $K_i > 330$. Namely, Eq. (2) can be used practically for sugars with $\kappa > 0.01$.

4. Conclusion

We previously proposed that Eq. (2) is available for the interaction between sugars and ConA, which has been associated with the immobilized glycoproteins. In this study, we examined further the binding of various sugars to ConA for understanding the theoretical background of Eq. (2). It was found that this equation can be used for quantitative analysis of the binding of sugars to ConA with K_i less than about 333.

Fig. 3. Simulation of the binding of sugar to ConA according to Eq. (1). Simulations were performed with K_i values of 33 (closed squares), 100 (open squares), 333 (closed triangles), 1000 (open circles), and 3333 (closed circles) at $k_-k_{tr}/\{k_+(R_0 - R_t)\} = 3 \times 10^4$ and $1/k_+ = 100$ according to Eq. (1). Regression lines according to Eq. (2) are also shown.

Fig. 4. Dependence of the intercept of Fig. 3 (ν) on K_i . Values of ν determined from the intercepts of the linear regression lines in Fig. 3 are plotted as a function of K_i . Symbols are as for Fig. 3.

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