Slow dissociation of 4-methylumbelliferyl β-D-galactosyl-(1⟶3)-*N*-acetyl β-D-galactosaminide from its complex with peanut agglutinin

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Interaction of peanut agglutinin with MeUmbβGalβ(1⟶3)GalNAc was followed with the stopped-flow technique. The mechanism is a simple bimolecular association with $k_+ = 7.1 \times 10^3\ M^{-1}.s^{-1}$ and $k_- = 0.24\ s^{-1}$ at 25°C. The very slow dissociation rate of the complex strongly supports earlier conclusions that the combining site of peanut agglutinin is complementary to the Galβ(1⟶3)GalNAc structure.

Lectin *Peanut* *Carbohydrate* *Binding site* *Difference absorption* *Stopped flow*

1. INTRODUCTION

Peanut agglutinin (PNA), the lectin from peanuts (*Arachis hypogaea*), can be used to probe cell surfaces and to separate lymphocyte subpopulations [1,2]. This follows from the ability of PNA to bind to the nonsialylated structure from some glyconjugates, β-D-galactopyranosyl-(1⟶3)-*N*-acetylgalactosamine [Galβ(1⟶3)GalNAc] that is α-*O*-linked to serine or threonine in glycoproteins. Indeed, PNA seems to possess an extended combining site [3–5], most complementary to Galβ(1⟶3)GalNAc. This disaccharide binds about 36-times better than Me β-Gal and 14-times better than methyl β-lactoside.

To obtain insight into the interaction of PNA with carbohydrates and cell surfaces, detailed thermodynamic and kinetic information has been obtained on the binding of methyl glycosides and PNA by ^{13}C-NMR and ultraviolet difference absorption techniques [5–8]. However, these studies employed carbohydrates showing affinities that are much lower than that of Galβ(1⟶3)GalNAc. Here, we use a derivative of this disaccharide. It is MeUmbβGalβ(1⟶3)GalNAc which undergoes discrete optical changes upon binding to PNA [9,10]. The data obtained here by stopped-flow kinetics are consistent with a simple bimolecular reaction. They demonstrate that the dissociation-rate parameter for the most complementary disaccharide derivative is at least 100-times slower than for the carbohydrates investigated to date.

2. MATERIALS AND METHODS

PNA, obtained by affinity chromatography [11], was a gift from Nathan Sharon. The protein was dissolved (17 mg/ml) in 0.05 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), 0.5 M $MgCl_2$, 0.1 mM NaN_3 (pH 6.9), dialyzed against the same buffer at 22°C to promote solubility [12]. Its concentration was determined at 280 nm, with <2% correction for stray light, using

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Abbreviations: Me α-Gal, methyl α-D-galactopyranoside; Me β-Gal, methyl β-D-galactopyranoside; MeUmbβGalβ(1⟶3)GalNAc, 4-methylumbelliferyl β-D-galactopyranosyl-(1⟶3)-*N*-acetyl-β-D-galactopyranosaminide; PNA, peanut agglutinin

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an absorption coefficient of 0.96 $mg^{-1}.cm^2$ and expressed as binding equivalents on the basis of M_r = 27500 for a promoter [13].

MeUmbβGalβ(1⟶3)GalNAc was a gift from Khushi L. Matta. Its concentration was determined at 318 nm with $\epsilon = 1.38 \times 10^4\ M^{-1}.cm^{-1}$. Me α-Gal was from Sigma (St Louis MO).

Stopped-flow kinetics were followed at 25°C with a Dionex apparatus, linked with an OLIS data collecting system. Absorption differences were measured at 334 nm [10] using a spectral band width of 4.5 nm. To obtain the reaction-rate parameters, a kinetic titration series was done under pseudo-first order conditions by mixing 16 to 24 μM MeUmbβGalβ(1⟶3)GalNAc with 104 to 622 μM PNA. The dissociation-rate parameter of the MeUmbβGalβ(1⟶3)GalNAc·PNA complex was confirmed by a direct determination, which involved mixing of 0.1 or 0.5 M Me α-Gal with a mixture of 23 μM MeUmbβGalβ(1⟶3)-GalNAc and 236 μM PNA.

3. RESULTS AND DISCUSSION

All progress curves in the two types of kinetic experiments were fully consistent with a single-exponential change. Formation of the MeUmb-βGalβ(1⟶3)GalNAc·PNA complex resulted in an increase of MeUmb absorption [10] (not shown). The observed reaction rate, k_{obs}, increased linearly as a function of PNA concentration (fig.1). This is consistent with an apparently simple bimolecular one-step reaction:

$$A + B \underset{k_-}{\overset{k_+}{\rightleftharpoons}} C$$

with the slope in fig.1 equal to $k_+ = 7.1 \times 10^3\ M^{-1}.s^{-1}$. This value is about 5-times smaller than for Me α-Gal, Me β-Gal and methyl β-lactoside [5–8] and about 10^5-times smaller than for a diffusion-controlled association. Slow binding of carbohydrates seems to be typical of other lectins such as concanavalin A [14–15] and those from the seeds of *Ricinus communis* [16], *Griffonia (Bandeiraea) simplicifolia* [17], *Triticum vulgaris* [18], and *Glycine max* (unpublished). These slow phenomena have been explained as being caused by a two-step mechanism: rapid and unobserved formation of a lectin–saccharide complex that undergoes a slow conformational change. In this respect, the low value of k_+ for MeUmb-βGalβ(1⟶3)GalNAc and PNA can reflect a rate-determining conformational change that is somewhat more pronounced than for the other sugars. In the time window accessible to the stopped-flow technique, PNA showed a single kinetic process with a concentration dependence corresponding to a simple bimolecular association. In particular, there is no evidence for 'sliding' of the disaccharide over the combining region as has been inferred from multiphasic kinetics for binding of disaccharides derivatives to concanavalin A [19,20] and wheat germ agglutinin [18].

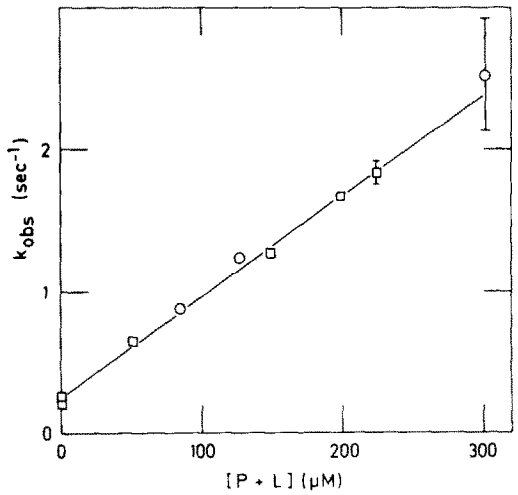

Fig.1. Determination of the reaction-rate parameters for binding of MeUmbβGalβ(1⟶3)GalNAc and PNA using the stopped-flow technique with PNA in excess. Experimental conditions are 25°C and pH 6.9. The observed reaction rates (k_{obs}: ○. av; 2–4 expt; □, av. 5–10 expt) are plotted against the sum of free concentration of reactants ([P + L]). The intercept corresponds to $k_{-1} = (0.24 \pm 0.01)\ s^{-1}$ and the slope to $k_{+1} = (7.1 \pm 0.2)\ 10^3\ M^{-1}.s^{-1}$. The data for [P + L] = 0 were determined independently like in fig.2.

The important finding with MeUmbβGalβ-(1⟶3)GalNAc is the extremely slow dissociation from its complex with PNA: the dissociation-rate parameter, $k_- = 0.24\ s^{-1}$ (fig.1, fig.2) is about 100-times slower than for Me α-Gal, Me β-Gal or methyl β-lactoside [6–8]. Most probably, this is a consequence of the complementary nature of the

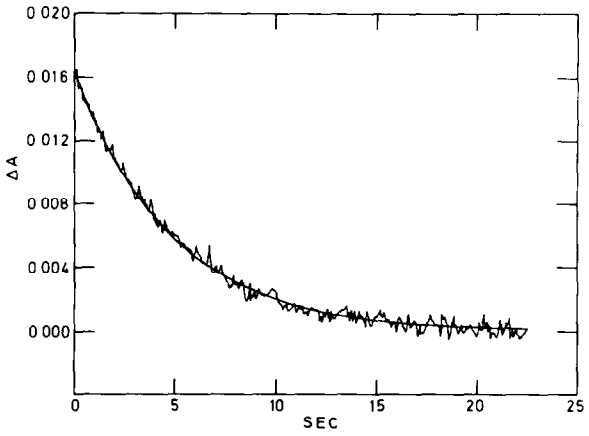

Fig.2. Direct determination of the dissociation-rate parameter k_- of the MeUmbβGal(1⟶3)GalNAc·PNA complex. A mixture of 23 μM MeUmbβGalβ(1⟶3)-GalNAc and 236 μM PNA sites was mixed with 0.5 M Me α-Gal. The average of 6 expt is shown together with the calculated monoexponential corresponding to $k_- = k_{obs} = 0.21\ s^{-1}$; $-\Delta A_{334} = 0.017$. As a control, 0.5 M Me α-Gal was substituted by 0.1 M Me α-Gal yielding $k_- = k_{obs} = 0.27\ s^{-1}$; $-\Delta A_{334} = 0.014$.

combining region for the Galβ(1⟶3)GalNAc structure, as deduced from the values of the binding enthalpy [5,9,10] and dissociation enthalpy [8]. For MeUmbβGalβ(1⟶3)GalNAc, the value of the kinetically determined association constant $K = k_+/k_- = 2.9 \times 10^4\ M^{-1}$ at 25°C agrees with $K = 2.8 \times 10^4\ M^{-1}$ determined at equilibrium at 25°C for the unsubstituted Galβ(1⟶3)GalNAc [5]. It seems to be the Galβ(1⟶3)GalNAc structure, rather than the MeUmb group, that causes a 100-fold decrease in dissociation rate since, in addition, this derivatisation influences the equilibrium value of K by a factor that is <2 (fig.1, [5,10,12]).

The situation observed with PNA resembles that reported for concanavalin A [21,22]: in a series of mono- and disaccharides and some of their *p*-nitrophenyl and MeUmb glycosides, it is the dissociation-rate parameter that determines the affinity. For concanavalin A, derivatisation of the monosaccharides does not affect the binding mechanism and has only minor effects on the reaction-rate parameters of carbohydrates with preferred binding configuration [21,22]. A similar situation can be expected with PNA.

4. CONCLUSION

Dissociation of MeUmbβGalβ(1⟶3)GalNAc from its complex with PNA is slower by 2 orders of magnitude than for other mono- and disaccharides [6–8]. This slow dissociation rate, $k_- = 0.24\ s^{-1}$, seems to be a consequence of the complementary nature of the PNA combining region and the Galβ(1⟶3)GalNAc structure.

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