DYNAMICS OF CARBOHYDRATE–LECTIN INTERACTION

The interaction *p*-nitrophenyl-β-D-galactose with a lectin from *Ricinius communis*

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Received 18 November 1977

1. Introduction

Lectin-induced agglutination is a complex process determined by several factors such as the nature of lectin (valency, binding constant) the properties of cell membrane (fluidity, distribution of lectin receptor sites) and the metabolic state of the cell (microvilli, microtubules, microfilament) [1–3]. In order to assess quantitatively the effect of each of these factors, one has to obtain the thermodynamic and kinetic parameters of the interaction of various lectins with simple sugars, glycolipids and glycoproteins. We have been trying to estimate these parameters, particularly from the kinetic analysis of the precipitin reaction of the glycoprotein–lectin and glycolipid–lectin systems in aqueous and lipid phases [4,5]. Here we wish to report our *T*-jump relaxation kinetic study on the binding of *p*-nitrophenyl-β-D-galactose (NPG) to RCA_1, a galactose specific lectin isolated from *Ricinius communis* beans and the turbidimetric data on the binding of galactomanan to RCA_1. It is also shown that the presence of a small amount of galactose or its derivatives decreases the initial rate of the precipitin reaction. The relevance of this finding to lectin receptor binding assay is also disscussed.

A part of this work was done at the Max-Planck Institute for Biophysical Chemistry, 3400-Göttingen-Nikolausberg, FRG

2. Materials and methods

p-Nitrophenyl-β-D-galactose from Koch Light and galactose from E. Merck were used. The isolation and purification of RCA_1 from a local variety of *Ricinius communis* beans were done as described [4]. Galactomanan A was obtained from Dr Rees as a gift [6]. Spectral measurements were carried out using Cary 118. Temperature jump relaxation measurements were made in fluorescence *T*-jump apparatus, using absorption mode as described [7].

3. Results and discussion

Figure 1 shows the spectra of *p*-nitrophenyl-β-D-galactose in the presence and absence of RCA_1. There is an increased absorption in the region of its maximum absorption in contrast to that observed for nitrophenyl-α-D-mannoside in the presence of concanavalin A (conA) [8]. This may be taken to indicate that local environments around active site of the two proteins differ from each other. The temperature dependence of such observed changes in the spectrum of NPG is used to estimate kinetic constants of its binding to RCA_1. The *T*-jump relaxation spectrum obtained for the mixture of RCA_1 and NPG at 312 nm is shown in fig.2. The plot of logarithm of

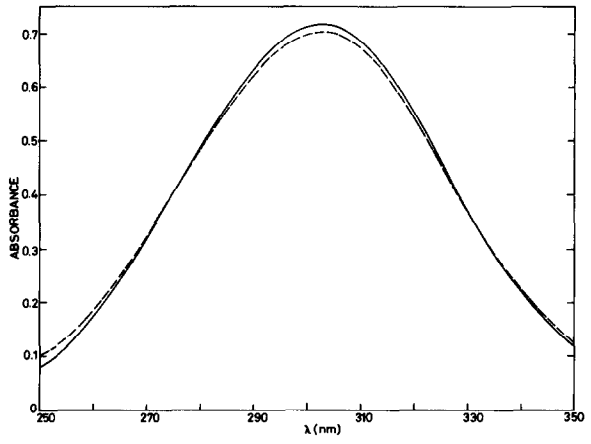

Fig.1. Spectra of NPG (6.4×10^{-5} M) in the presence of RCA_1 (1.13×10^{-6}) (—) and its absence (– – –).

the amplitude against time was completely linear and from the slope, the relaxation time, τ was calculated. For a bimolecular reaction of the type

$$RCA_1 + NPG \underset{k_D}{\overset{k_R}{\rightleftharpoons}} RCA_1 - NPG$$

the reciprocal of the relaxation time, $1/\tau$ is given by the equation [9].

$$1/\tau = k_R (\bar{C}_p + \bar{C}_L) + k_D \qquad (1)$$

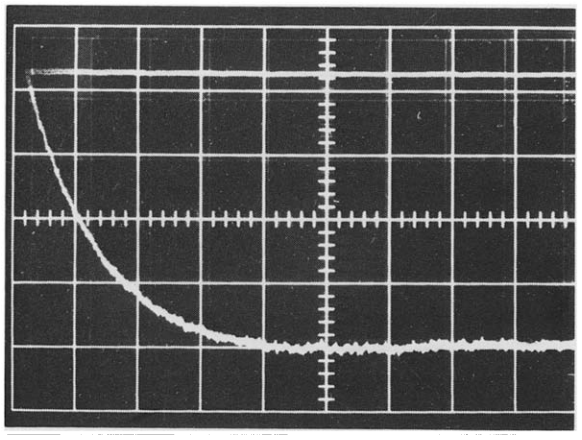

Fig.2. Typical oscillogram of a temperature jump relaxation curve for a mixture of RCA_1 (7.1×10^{-6} M) and NPG (1.4×10^{-4} M) in 50 mM phosphate buffer + 0.2 M NaCl at pH 6.8, sensitivity 10 mV/cm, time 5 msec/cm.

where k_R and k_D represents rate constant of the formation and dissociation of the complex respectively. $\bar{C}_p$ and $\bar{C}_L$ are the equilibrium concentration of protein, RCA_1 and ligand, NPG, respectively. Figure 3 shows the plot of $1/\tau$ (obtained from the average of three determinations with a variation of ± 5%) against sum total of $\bar{C}_p$ and $\bar{C}_L$. The slope and intercept give the value of k_R and k_D as $(4.5 \pm 0.2) \times 10^5$ M^{-1} s^{-1} and 30 ± 3 s^{-1} at 28°C. The corresponding value for the binding of *p*-nitro-α-D mannopyronoside to concanavalin A which binds specifically to mannose and has four binding sites are reported as 5.4×10^4 M^{-1} s^{-1} and 6.2 s^{-1} at 25°C [10,11].

The rate data reported here for RCA_1 is quite different from those expected for diffusion controlled reaction. They are however comparable to those of conA [11]. That the forward rate constant k_R of RCA_1 is higher than that of conA suggests that it should act as a better agglutinating agent even though it is a divalent lectin. Literature data show that it can agglutinate certain cells at much lower concentration than that required for conA even though conA is tetravalent and is capable of recognising internal sugar residues [12].

The association constant of the interaction of RCA_1 with NPG is then calculated as 1.5×10^4 M^{-1} (28°C) from the measured ratio k_R/k_D. This value is in agreement with that obtained from equilibrium dialysis data (1.68×10^4 at 25°C) [13] and from relative rate of formation of precipitin complexes in the presence of NPG (see below). Figure 4 shows the changes in absorbance (i.e., turbidity) at 320 nm

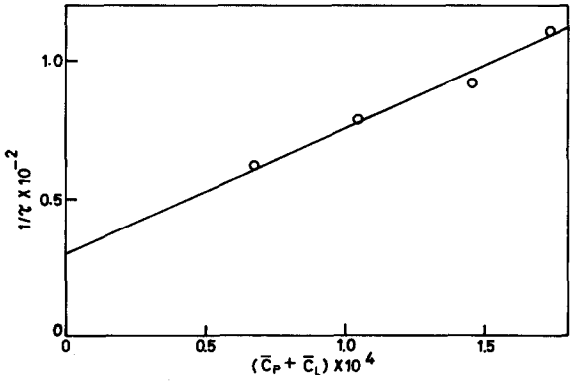

Fig.3. Plot of $1/\tau$ against total concentration of RCA_1 ($\bar{C}_p$) and NPG ($\bar{C}_L$).

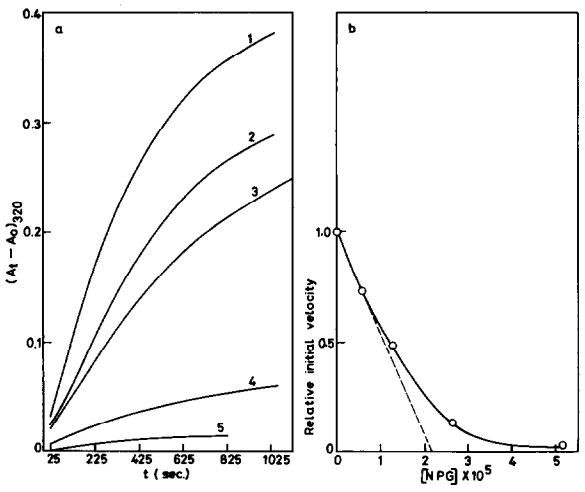

Fig.4a. Time dependence of precipitin reaction between galactomanan A (0.11 mg/ml) and RCA_1 (0.84×10^{-6} M) in the presence of varying amount of NPG. (1) 0.0; (2) 5.6×10^{-6} M; (3) 13.1×10^{-6}; (4) 26.2×10^{-6}; (5) 52.4×10^{-6}. Fig.4b. Plot of relative initial velocity against the concentration of NPG corresponding to the data shown in fig.4a.

when constant amount of galactomanan A is added to a solution of RCA_1 containing different amounts of NPG. It is interesting to note that both the rate and the extent of precipitin reaction decrease with increasing concentration of NPG. Evidently this is resulted from a decrease in the available concentration of RCA_1 for the reaction with galactomanan. The rate and mechanism of the precipitin reaction as measured by turbidity is quite complex, and will be discussed in detail in a separate publication. It is determined not only by absolute concentration of reactants but also by their ratios. The exact mechanism is, however, determined by relative magnitude of dissociation rate constants of lectin–sugar complexes and also by the product of the rate constant of aggegration giving rise to turbidity and the concentration of galactomanan. The rate constant of aggregation is determined by the structural complexity of galactomanan. Since the reaction of simple galactose derivatives like NPG, with RCA_1 can occur more rapidly, one can assume that there is an instantaneous equilibrium between free and bound NPG. The observed decrease in the rate of precipitin reaction is due to a decrease in the concentration of RCA_1 by a factor $(1 + KH)^{-1}$ [14], where K is the association constant of binding of hapten, i.e., NPG to RCA_1 and H is the total concentration of NPG in mol/litre. The fig.4b shows the plot of relative initial rate against the concentration of NPG. From the initial slope, the binding constant is calculated as 4.5×10^4 M^{-1} at 20°C. This value is in good agreement with that obtained from equilibrium dialysis [13] and relaxation kinetic experiments. The calculated association constant from the decrease in the rate of precipitin reactions in the presence of lactose is in good agreement with that obtained from the equilibrium method [4].

Thus the measurement of initial rate of precipitin reaction in presence of hapten provides a rapid excellent method for the estimation of binding constant. In order to give corrections for non-specific binding, cell surface receptors are usually assayed in presence of a hapten. Since the presence of hapten decreases the rate of receptor–ligand binding, as revealed by the present study, one may go wrong in the estimation of the number of specific receptors for the ligand, especially when the binding data are obtained after a short period of incubation of reaction mixture before equilibrium is attained [15]. It is quite evident that a suitable kinetic assay procedure is to be developed for the estimation of the number of cell surface receptors.

Acknowledgements

We thank the Council of Scientific and Industrial Research for financial support, the Max-Planck Society for the advancement of Science, FRG and Professor M. Eigen for the award of a fellowship to one of us (S.K.P.) and Dr Rees for the gift of galactomanan.

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