Nature of the interaction between *Ricinus communis* agglutinin and blood cells

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Received 13 July 1984

Binding of *Ricinus communis* agglutinin (RCA 120) to carbohydrate receptors of human lymphocytes and erythrocytes is enthalpically driven. As in the case of simple saccharides, the ΔS contribution is always unfavorable to the interaction. This result is different from that observed for other lectins and might indicate that hydrophobic interactions do not play a dominant role in binding of RCA 120 to cell surfaces.

Ricinus communis *agglutinin* *Human lymphocyte* *Lectin–cell surface interaction*

1. INTRODUCTION

The binding of lectins to cells through receptors is the preliminary requirement of any biological activity attributed to these substances [1]. Binding parameters have been measured for many lectin–cell interactions. However, the molecular features of this binding are still largely unknown, both with regards to their biochemical characteristics, such as carbohydrate structures and the distribution and mobility of the receptors in the membrane, and their physical aspects (e.g., interaction characteristics and size of the lectin binding sites) [2].

Human lymphocytes, which bind lectins readily and are involved in many important immunological processes, constitute a convenient system for studying the interaction between cells and ligands such as hormones, toxins or drugs and the subsequent effects [3].

This paper deals with the thermodynamic aspects of the interaction between *Ricinus communis* agglutinin (RCA 120, M_r = 120000), a lectin which specifically binds D-galactosides [4], and its receptors on normal human lymphocytes from peripheral blood.

2. MATERIALS AND METHODS

RCA 120 was purified from *R. communis* seeds according to the method of [5] and labeled with [^{14}C]acetic anhydride according to [6]. [^{14}C]RCA 120 was repurified by gel filtration on Sephadex G-200 in 0.05 Tris-HCl buffer, pH 7.4. The [^{14}C]RCA 120 homogeneity was checked by polyacrylamide gel electrophoresis in the absence and presence of SDS and β-mercaptoethanol, and its activity was identical to that of the native lectin, i.e., the minimum hemagglutinating dose was 1.7 μg/ml when tested with human O^- erythrocytes. The specific activity of [^{14}C]RCA 120 was 1400 dpm/μg.

Lymphocytes were purified from 'buffy coats' by centrifugation on a Ficoll-Hypaque gradient according to [7]. Cell viability, estimated by trypan blue exclusion, was more than 95%.

Lymphocyte surface glycoproteins were released by mild trypsin treatment followed by maleylation as reported [8].

The interaction between RCA 120 and lymphocyte surface glycoproteins was measured by several series of Sephadex G-200 gel filtrations of different mixtures of [^{14}C]RCA 120 and glycoproteins, as described [9]. Results were plotted according to Scatchard [10] and allowed estimation of the receptor numbers and affinity constants of this system at 4 and 20°C.

The interaction of [^{14}C]RCA 120 with fresh lymphocytes and erythrocytes was measured at 4, 20

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00145793/84/$3.00

and 29°C according to [11] and the results were treated as above.

The interaction of [^{14}C]RCA 120 with cells and solubilized receptors was reversible under our experimental conditions, since the addition of 0.1 M lactose to the reaction medium abolished lectin–receptor binding almost completely (>95%).

Regression curves were computed by the least-square method with a Hewlett-Packard 9815A calculator.

3. RESULTS

The Scatchard representation of the binding of [^{14}C]RCA 120 to solubilized receptors from human lymphocytes is linear [8]. The intercept of the lines with the abscissa allowed estimation of the number of receptors. Values were calculated assuming an RCA 120/receptor ratio of 1 [8].

The number of receptors in solution was not very different at 4 and 20°C, i.e., 7 and 4.5×10^4 per cell, respectively (fig. 1).

[^{14}C]RCA 120 affinity for solubilized receptors was higher at 4°C than at 20°C. The affinity constants were 4.7 and 1.6×10^6 M^{-1}, respectively (table 1).

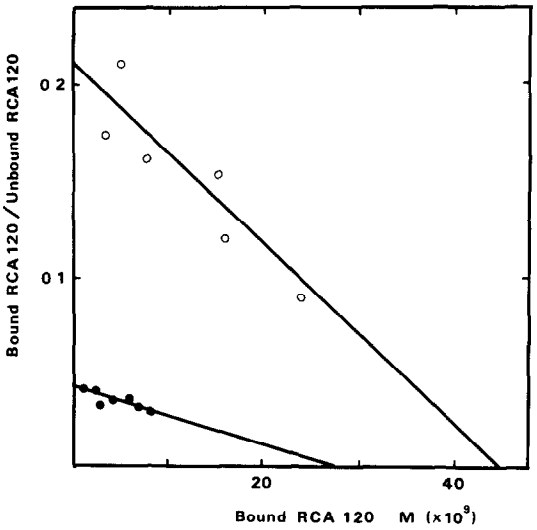

Fig. 1. Interaction of RCA 120 with solubilized lymphocyte receptors. 100 μl trypsinate (4 mg proteins/ml) were incubated with increasing amounts of [^{14}C]RCA 120 (1–20 μg/100 μl) for 15 min at 4 (○) or 20°C (●) and loaded on a Sephadex G-200 column (50×1 cm) equilibrated in 0.05 M Tris-HCl (pH 7.4). Fractions (0.5 ml) were collected and counted for their radioactivity [9].

For the binding to whole lymphocytes, biphasic curves with an upward concave slope were obtained (fig. 2A). This suggests the existence of at least two receptor populations referred to below as low- and high-affinity receptors. Their respective numbers were 5.4 and 1.2×10^7 per cell; these numbers were higher than those found at both temperatures for solubilized receptors. For whole lymphocytes, the affinity was determined at 3 temperatures (4, 20 and 29°C, table 1); the corresponding values decreased with the increase in temperature for both high- and low-affinity receptors.

The values for low-affinity receptors, at 4 and 20°C, 4.7 and 1.9×10^6 M^{-1}, were close to those found for solubilized receptors at the same temperature.

In the concentration range studied, the results with the fresh erythrocyte receptors (fig. 2B) were of the same order of magnitude (table 1) and again the affinity constant decreased between 4 and 29°C.

These affinity values allowed calculation of the thermodynamic parameters of the interaction between RCA 120 and the lymphocyte receptors. From van 't Hoff plots, the enthalpy variations were −10.8 kcal/mol for solubilized receptors and −12.3 (low affinity) and −14.0 (high affinity) kcal/mol for whole cells (fig. 3). For fresh erythrocytes, ΔH was −11.6 kcal/mol.

These values are close and are all negative. Furthermore, they are of the same order of magnitude as those measured for binding of RCA 120 to simple carbohydrates, D-galactose and lactose (table 1). Entropy variations, calculated from these results were negative in all cases.

4. DISCUSSION

Receptors for RCA 120 on the surface of peripheral blood normal human lymphocytes are glycoproteins and glycolipids. Each of these glycoconjugates is responsible for about half the binding of this lectin to lymphocyte membranes [11].

When lymphocytes were treated with trypsin, the receptors thus obtained were almost exclusively

glycoproteins. Gel filtration experiments on Sephadex G-200 showed that isolated receptors binding [^{14}C]RCA 120 exhibited high molecular mass, since radioactivity was eluted at the void volume of the column [8]. Furthermore, only traces of glycolipids were released in the cell trypsinate as checked by thin layer chromatography (not shown). In any case, lectins and glycolipids interact very poorly in aqueous solution and the latter need to be included in liposomes to exhibit any lectin binding activity [12].

The biphasic Scatchard plots obtained here for RCA 120 binding to whole lymphocytes were more probably due to the presence of a heterogeneous receptor population than to negative cooperativity, which requires receptor mobility, since the same results as those of the present work were obtained earlier [11] with formaldehyde-fixation of lymphocytes, which precludes such mobility. The mild trypsin treatment released a small amount of apparent low-affinity receptors (table 1), although one cannot exclude the possibility that these might have been high affinity receptors whose affinity decreased with the loss of membrane environment.

The main sources of complex stability in the binding of lectins to carbohydrates seem to be hydrogen bonding and charge transfer interactions [13]. This binding is enthalpically driven for RCA

Table 1

Thermodynamic parameters of the RCA 120-ligand interactions

	Temperature (°C)	K_a $M^{-1}(\times 10^{-3})$	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS (cal/mol/°K)
D-Galactose[a]					
calorimetry	25	2.6	−4.6	−12.3	−26.3
equilibrium	4	7	−4.9	−10.2	−19.3
dialysis	18	2.8	−4.6		
Lactose[a]					
calorimetry	25	23.5	−6.0	−11.0	−17
equilibrium	4	74	−6.2	− 7.9	− 6.3
dialysis	25	26.7	−6.0		
Solubilized	4	4700	−8.4	−10.8	− 8.7
receptors[b]	20	1600	−8.3		
Lymphocytes[c]					
low	4	4700	−8.4		
affinity	20	1900	−8.4	−12.3	−14.0
	29	680	−8.0		
high	4	74000	−9.9		
affinity	20	20000	−9.8	−14.0	−14.8
	29	8700	−9.6		
Erythrocytes[d]	4	4000	−8.3		
	20	1200	−8.1	−11.6	−11.9
	29	700	−8.0		

[a]From [14]
[b]Affinity constants determined from fig. 1
[c]Affinity constants determined from fig. 2A
[d]Affinity constants determined from fig. 2B

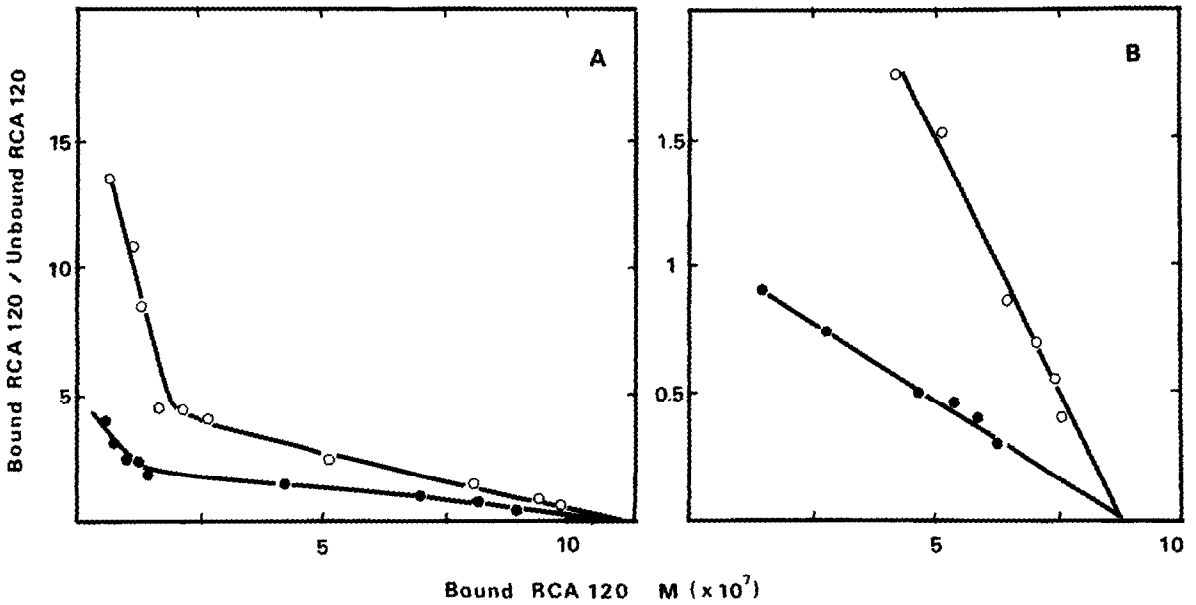

Fig. 2. Interaction of RCA 120 with blood cells: (A) human lymphocytes, (B) human O^- erythrocytes. Cells (1.25×10^7/ml) were incubated at 4 (○) or 20°C (●) with increasing concentrations of [^{14}C]RCA 120 (lymphocytes 7.5–200 μg/ml, erythrocytes 50–200 μg/ml) [11].

120 [14] and RCA 60 [15], the two closely related lectins of *R. communis* [16], as well as for many other lectins. Although, from a thermodynamic point of view, hydrophobic interactions cannot play a primary role in this binding, they probably modulate the specificity of the interaction through complementary hydrophobic regions of the lectin binding site and of the saccharide surface [17]. For instance, a D-galactose unit can offer a substantial hydrophobic surface in its preferred conformer [18].

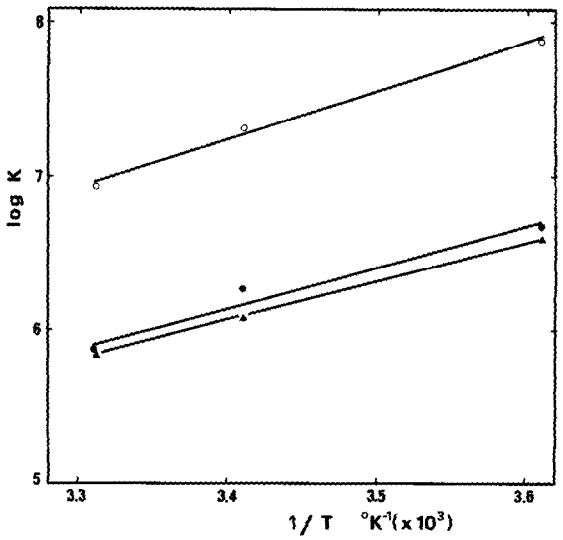

Fig. 3. Van 't Hoff plot for the binding of RCA 120 to cells: (○) lymphocytes low-affinity receptors, (●) lymphocytes high-affinity receptors, (▲) erythrocytes.

Interactions between lectins and cell surfaces are generally entropically driven, and a higher affinity constant can be measured at and above 20°C compared to 4°C. This is true for the interaction with many cell types of Concanavalin A [19–23], *Phaseolus vulgaris* agglutinin [24], the N-blood group specific lectin from *Vicia graminea* (M.J. Prigent, personal communication) and probably wheatgerm agglutinin [25,26]. The affinity of *Lens culinaris* agglutinin for erythrocytes is higher at 4°C but ΔH and ΔS contribute equally to ΔG, the Gibbs free energy term of the interaction [27]. These results were attributed to hydrophobic interactions between lectins and cell surfaces and hydrophobic areas have been proved to exist in the sugar binding sites of Concanavalin A [28] and wheatgerm agglutinin [29], since the fluorescence of their specific 4-methylumbelliferyl-glycosides is quenched upon their binding to these two lectins

[30]. Furthermore, the presence of an additional hydrophobic binding site, independent of the sugar binding one, has been demonstrated for Concanavalin A, where it can bind indoleacetic acid [31], and for other lectins [32].

Like RCA 60, RCA 120 does not affect the fluorescence intensity of 4-methylumbelliferyl-β-D-galactopyranoside [33]. Binding of RCA 60 to Hela cells has been reported to be stronger at 4 than at 37°C [34], and study of the thermodynamic properties of RCA 60 and RCA 120 adsorption on Sepharose 4B shows that, for both lectins, the ΔS contribution was unfavorable to the interaction [35].

From the results, it is clear that RCA 120 binding to lymphocytes and erythrocytes is different from that observed for other lectins, since it is enthalpically driven for both isolated receptors and fresh cells. As in the case of simple saccharides [14], ΔS is always unfavorable to the binding (table 1), which therefore might not comprise hydrophobic interactions as the main forces.

ACKNOWLEDGEMENTS

The authors are very grateful to Dr Reviron (Centre de Transfusion Sanguine, Hôpital Saint-Louis) for supplying them with blood. This work was supported by grants from the INSERM (CRL no. 79-5-162-3), the CNRS (L.A. 293), the Universités Paris V and Paris VII.

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