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Note

General method for the study of cell receptor site–lectin interactions

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A gel filtration technique has been developed for the study of hormone receptor sites (*e.g.*, insulin, follicle-stimulating hormone, thyroid hormone)[1–3]. This method allows the characterization of reversible complex formation between hormones and their cellular receptor sites or carrier proteins. It has also been applied to the fixation of drugs to plasma proteins[4].

Similarities exist between some hormone receptor sites and the lectin sites: membrane localization, glycoprotein structure[5,6]. For instance, it has been observed that the binding of concanavalin A and wheat germ agglutinin to adipocyte insulin receptors induces the same intracellular metabolic modifications as the hormone[7].

Consequently, a method derived from hormone receptor studies has been adapted to lectins. Some parameters of the lectin–receptor site binding have been determined. Glycoprotein material, released and solubilized from the outer surface of normal human lymphocytes was used so as to avoid all possible interactions with the vicinal membrane components. Some of these results are compared with those obtained with intact lymphocytes.

MATERIALS AND METHODS

The studied macromolecules were obtained from normal human lymphocytes as previously described[8]. Sephadex G-200 (Pharmacia, Uppsala, Sweden) was swollen in two different buffers, namely: A, 0.05 *M* Tris–HCl, pH 7.5; and B, 0.05 *M* Tris–HCl (pH 7.5) + 0.1 *M* lactose. The gel was poured into either an analytical glass column (65 × 1 cm) or a preparative one (100 × 2.2 cm). The lectin *Ricinus communis* (var. *sanguineus*) agglutinin (RSA 120), prepared in the laboratory in a homogeneous state, was labelled with [^{14}C]acetic anhydride, according to the method of Miller and Great[9], and repurified by gel filtration on a Sephadex G-200 column (100 × 2.2 cm) in buffer A. After concentration the biological activity of the lectin, *i.e.*, the erythrocyte agglutinating activity, is maintained. Aliquots of the labelled lectin were stored at −20° and thawed before each experiment. The homogeneity of the preparation was controlled by polyacrylamide gel electrophoresis with or without sodium dodecyl sulphate according to Davis[10] or Rodbard and Chrambach[11], respectively. A specific activity of 8 × 10^5 cpm per mg of protein was obtained.

The determination of the binding parameters on intact lymphocytes was performed according to the method of Sharif *et al.*[12].

The radioactivity content of the fractions was counted in an Intertechnique spectrometer with ACS (Amersham-Searle, Amersham, Great Britain) as scintillation cocktail. Results were plotted according to the method of Scatchard[13].

RESULTS

Evidence for the presence of lectin receptor sites in a solution

A mixture of the solubilized membrane material and [^{14}C]RSA 120 was applied on a Sephadex G-200 column and eluted with buffer A. The shift of a part of the ^{14}C radioactivity towards higher molecular weights suggested the formation of a complex between the receptor sites and lectin. The complex was eluted in the void volume of the column, determined by blue dextran exclusion, while free lectin was retained as the lectin alone (Fig. 1).

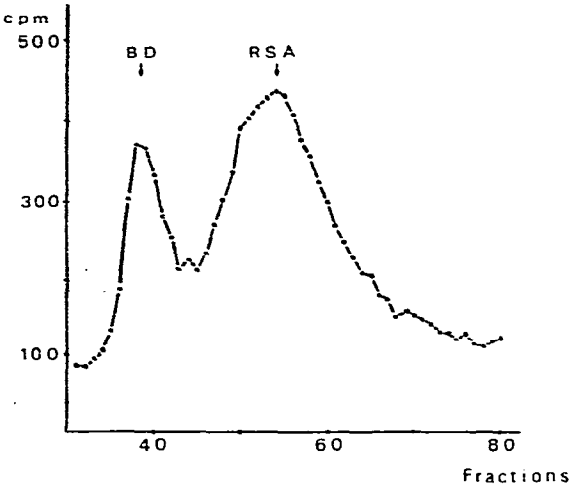

Fig. 1. Sephadex G-200 filtration (column, 65 × 1 cm) in buffer A of a mixture of [^{14}C]RSA 120 (7250 cpm) and solubilized surface material (120 μg protein). Fractions of 0.5 ml were collected and counted for their ^{14}C content as described in Materials and methods. BD = Blue Dextran.

Study of the inhibition of the lectin binding

When the same experiment was done in buffer B, the presence of lactose, which is a haptenic inhibitor of RSA 120, prevented the complex formation and all the [^{14}C]lectin emerged from the column within the normal elution volume (Fig. 2). The formation of a specific complex between RSA 120 and lymphocyte-solubilized receptor sites was thus demonstrated.

Determination of binding parameters

Since the specificity of the binding has been demonstrated, a series of gel filtrations was performed using a constant amount of solubilized material and increasing amounts [^{14}C]RSA 120. The amounts of bound and unbound radioactivity determined from the elution patterns and plotted according to Scatchard allowed the calculation of the affinity constant and the amount of lectin bound at saturation

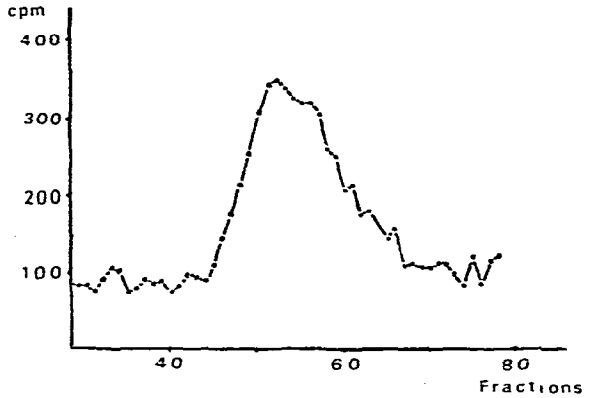

Fig. 2. The same experiment as described in Fig. 1 but carried out in presence of buffer B. Before loading, the sample was incubated with 0.2 *M* lactose.

(Fig. 3). The affinity constant of RSA 120 for the lymphocyte-solubilized receptor sites at 4° was $3.5 \times 10^6\ M^{-1}$. If we assumed that the ratio between the amounts of receptor site and lectin was 1:1, the amount of solubilized receptor sites could be determined as 3.4×10^{13} sites per ml. Since 2.5×10^7 cells were treated the trypsin digestion released 1.3×10^6 sites per cell. The determination of the same parameters with intact lymphocytes gave $2.6 \times 10^6\ M^{-1}$ for K_a and 3×10^7 receptor sites per cell. The trypsin treatment released only 4% of the membrane receptor sites.

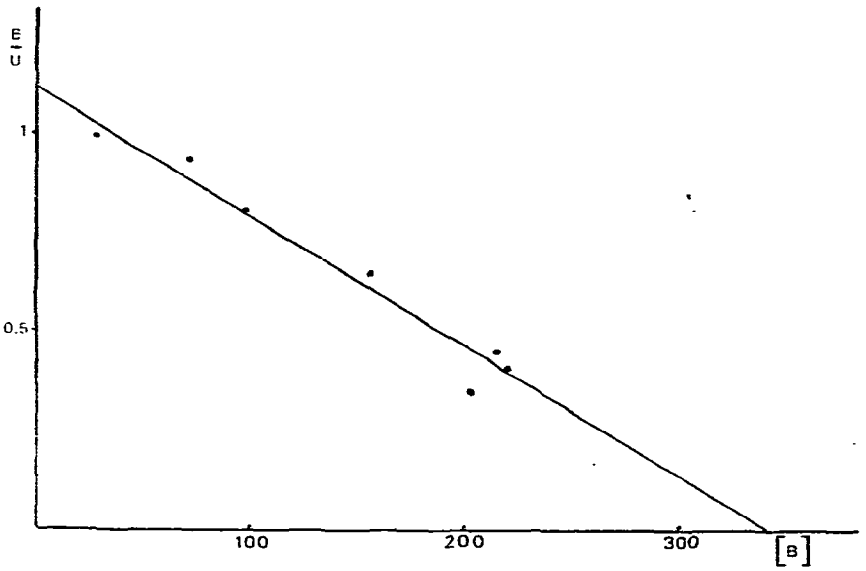

Fig. 3. A plot according to Scatchard of the data obtained from a series of Sephadex G-200 filtrations performed with a constant amount of solubilized lymphocyte receptor sites and increasing quantities of [^{14}C]RSA 120. Bound (B) and unbound (U) RSA 120 were determined by adding the ^{14}C counts in the respective peaks. On the abscissa axis, lectin concentrations are expressed in $10^{-9}\ M$. The data were processed by the least-squares method.

The affinity constant for intact cells and solubilized material were similar; thus the lymphocyte receptor sites exhibited the same behaviour in both cases, and would react independently of other membrane components.

Purification of solubilized receptor site

This method can be used quantitatively with a large Sephadex G-200 column (100 × 2.2 cm). A pattern similar to Fig. 1 was obtained. The radioactive peak containing the complexed receptor sites was easily separated from the nonactive membrane components. The labelled lectin should be further removed from the receptor sites by haptenic inhibitor competition.

DISCUSSION

The gel filtration technique has been widely applied to the study of complex formation between various interacting components. It seems to be a very convenient method for investigating the binding of lectins to solubilized cell receptor sites and appears more suitable than the usual cellular tests, *i.e.*, haemagglutination, haptenic inhibition, modification of cell growth, etc.

Some advantages and applications of this method are detailed here:

(1) Only very small amounts of the active compounds are required for these experiments. About 1 μg of solubilized lymphocyte receptor sites has been used for each gel filtration experiment. This is 100–200 times lower than the quantity needed for the haemagglutination inhibition tests, which are generally performed to check the ability of a substance to bind to a lectin.

(2) This technique is quantitative and more specific than haemagglutination tests. Since it is direct, it does not necessitate that the receptor sites studied share the same carbohydrate determinants as the erythrocyte membrane.

(3) When lymphocyte cultures are performed some parameters are difficult to handle and control, *e.g.*, culture media, presence of calf or autologous sera, pH and temperature. Moreover, in these cell techniques, large amounts of solubilized active compounds are needed and limit the use of cell tests.

(4) The lectin–receptor site binding can be easily inhibited. As described, the presence of a disaccharide inhibitor of the lectin[14] in the elution buffer partially or completely prevents the formation of a complex. Furthermore, the use of substances labelled with different isotopes allows an investigation of the competition between two lectins or any other ligand for the same receptor sites.

(5) This method can be used in conditions which are not suitable for cellular tests: pH, non-isotonic salt concentrations, temperature, presence of detergents which do not impede the gel filtration. Thus, interactions between RSA 120 and the surface glycoproteins of Zadjela's hepatoma cells have been studied in presence of a detergent (sodium deoxycholate) using this method[15].

These examples demonstrate the simplicity and versatility of this method for the study of the receptor site–lectin binding. Only the preparation of a pure radiolabelled lectin is needed. Comparison of the intact cell–lectin binding with the solubilized receptor site–lectin binding allows a definition of the nature of this interaction.

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