APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY 7, 71–74 (1982)

Partition Affinity Ligand Assay (PALA)

A Simple Binding Assay Procedure

TORBJÖRN G. I. LING* AND BO MATTIASSON

Pure and Applied Biochemistry, Chemical Center, University of Lund, PO Box 740, S-22007 Lund, Sweden

Accepted February 18, 1981

Abstract

In a competitive binding assay, the ligand to be quantified competes with a fixed amount of labeled ligand for the sites on a limiting amount of binding protein. The amount of label bound is therefore dependent on the ratio between native and labeled ligand. In a binding assay, one must separate the free ligands from bound. The better the separation, the higher the sensitivity of the assay. But effective methods are often laborious and time-consuming and thus we have developed a novel approach, the Partition Affinity Ligand Assay (PALA).

Index Entries: Partition affinity ligand assay; affinity ligand assay, by partition separation; ligand assay, by partition affinity chromatography; assay, partition ligand affinity; immunoassay, by partition affinity chromatography; digoxin, partition affinity ligand assay of.

Introduction

We present here a new approach to competitive binding assay based on the separation by partitioning of bound from free ligands in an aqueous two-phase system. First, the sample to be analyzed is mixed with a fixed amount of labeled ligand, after which specific antibodies are added. After binding has taken place for a fixed period of time, a well-mixed phase system is added. The two-phase system is

0273–2289/82/0912–0071$02.00

formed by mixing a solution of poly(ethyleneglycol) (molecular weight, 4000) with a magnesium sulfate solution. The mixture is then shaken vigorously on a vortex-type mixer for 10 s. Phase separation takes place within a few (3–5) minutes. In order to get an operating analytical procedure in a two-phase system, the free and bound ligands must partition to different phases. We found, however, that the partion behavior of many binding proteins does not allow an adequate separation, especially in reactions between two proteins. This problem was solved by modifying the binding protein and is illustrated here by two examples.

Experimental Procedure and Discussion

An assay for carbohydrates was done with the lectin from *Canavalia ensiformis* (con A) as a binding protein (*1*). The glucoenzyme peroxidase from horseradish was used as an enzyme-labeled carbohydrate ligand. This protein partitions to the salt-rich bottom phase (Fig. 1a). To achieve separation and sufficient analytical resolution, the lectin must partition to the poly(ethyleneglycol)-rich top phase. This was achieved by changing the surface properties by covalently attaching poly(ethyleneglycol) molecules to amino groups on the protein. When the

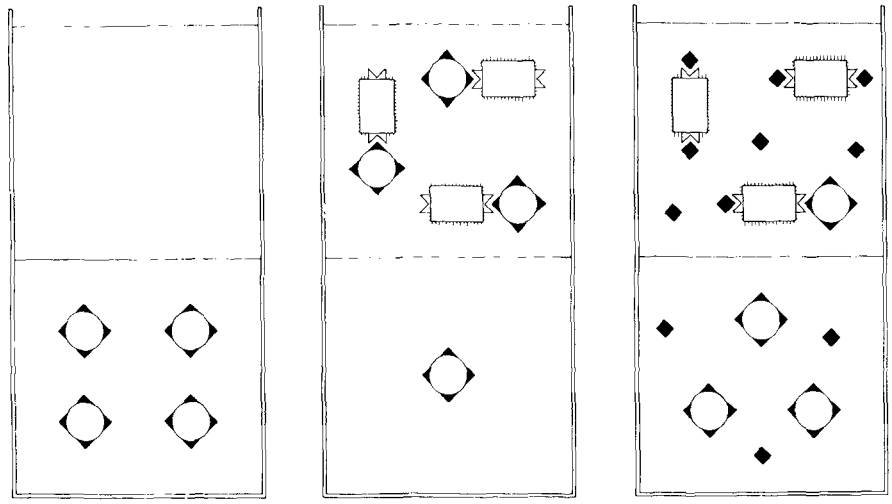

Fig. 1a. Peroxidase from horseradish partitions to the salt-rich bottom phase in an aqueous two-phase system containing 13.5 % (w/w) poly(ethyleneglycol) (molecular weight, 4000) and 13.5 % (w/w) $MgSO_4 \cdot 7H_2O$ and 10 mmol/L tris(hydroxymethyl)aminomethane, pH 7.00. Fig. 1b. Con A with attached poly(ethyleneglycol)molecules partitions to the top phase. When modified con A binds to the carbohydrate moiety of the enzyme, the complex goes to the top phase and thus the enzyme is transported over the phase boundary. Fig. 1c. Free carbohydrate molecules compete with the glucoenzyme for the binding sites on con A and thereby inhibit the transportation.

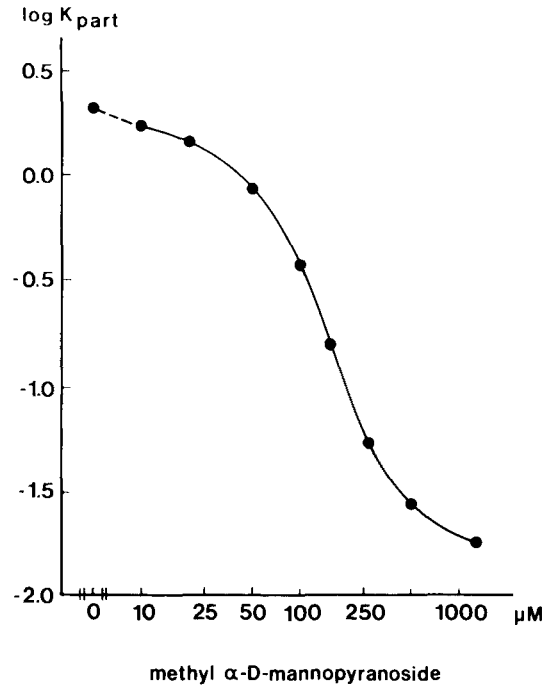

Fig. 2. Calibration curve for the system described in Fig. 1c. Log K_{part} (i.e., the ratio between the top and bottom phases, respectively) of the enzyme activity is plotted against the concentration of methyl α-D-mannopyranoside in the system.

modified lectin is incubated with the enzyme, it can transport bound enzyme molecules to the top phase (Fig. 1b). If free carbohydrates with ability to bind to the lectin are added to the system, they will displace some of the bound enzyme molecules, i.e., a competitive binding situation is achieved (Fig. 1c). A calibration curve for methyl α-D-mannopyranoside is shown in Fig. 2. The substrates for the enzyme reaction were added together with the phase contituents. Directly after the reaction mixture has been mixed, it was poured into spectrophotometer cuvets and the product formed in the bottom phase was read either kinetically or after a preset time. The operational concentration range was 25–1000 µmol/L. This is not as low as that of enzyme immunoassays using antibodies as binding proteins and depends on the association constants of antibodies being around 10^8 L/mol compared with 2×10^4 L/mol for the interaction between con A and carbohydrates.

In another assay digoxin was quantified (*2*). Digoxin is a frequently used cardioactive drug in the treatment of patients with cardiac insufficiency. It is, however, very toxic and has a narrow therapeutic zone, so it is essential to monitor the concentration in serum closely. Digoxin was found to partition to the top phase so that the specific antibodies used as binding proteins had to partition to the bottom phase. The native antibodies show a rather even distribution between the phases, but the difference was sufficient for a sensitive assay for digoxin. However, when antibodies bound to small Sephadex particles were used, a more favorable partitioning was obtained and thus better resolution. The partitioning of the reactants was thus reversed when compared to that in the carbohydrate assay. Iodine-125

labeled digoxin was used as a labeled ligand. After separation had taken place, a sample was removed from the top phase for determination of the radioactivity.

The operational concentration range was 1–8 nmol/L, which is the same as that of a commercial kit (from Pharmacia Diagnostics) and the coefficient of correlation between the two methods was 0.979. The total time of an assay using the PALA procedure could be as short as 15 min, but for practical reasons 30 min was used. Although the drastically shortened reaction time and the simplified washing procedure, the accuracy was comparable to that of the conventional radioimmunoassay.

Acknowledgment

This project was supported by the National Swedish board for Technical Development and Pharmacia Diagnostics.

References

1. Mattiasson, B., and Ling, T. G. I., *J. Immunol. Meth.* **38,** 217 (1980).
2. Mattiasson, B., *J. Immunol. Meth.* **35,** 137 (1980).