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THE AVIDIN-BIOTIN COMPLEX IN SOLID PHASE RADIOIMMUNOASSAYS

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A solid-phase radioimmunoassay is described in which biotin conjugated antibodies are used to replace ^{125}I labeled antibodies to individual antigens. The amount of antigen present is subsequently determined by the binding of ^{125}I-labeled avidin. This method is appealing for a variety of reasons. (a) Only one ^{125}I-labeled protein (avidin) need be prepared and characterized for all affinity systems. (b) There is no need to purify individual antibodies. (c) Biotin can be attached to antibodies under mild conditions. (d) The size, physical characteristics, and biological activity of the biotin-derived antibody are only nominally affected. (e) The biotin-avidin complex is of exceptionally high affinity and stability. (f) Introduction of biotin groups into the antibodies leads to amplified radioactive tracer binding. (g) Avidin and biotin are commercially available.

INTRODUCTION

Recently, we described several uses of the strong interaction system of avidin-biotin (association constant 10^{15} M) in biology and protein chemistry (1,2). To date, the avidin-biotin complex has been utilized for labeling of cell surface membrane components (3,4), for labeling and localization of cell surface receptor by a technique now known as affinity cytochemistry (5), for gene enrichments and visualization (6), and for immobilization and isolation of peptides and proteins (2). The use of this system for diagnostic purposes was described by us in 1972. Thus biotin modified bacteriophages were inactivated with avidin (7), similar to the inactivation of antigen modified bacteriophage with antibodies. In some cases, the bacteriophage technique approached the sensitivity of radioimmunoassay (RIA).

In the following, we describe the use of the avidin-biotin complex in solid-phase radioimmunoassay. The applicability of this system is demonstrated with ferritin, avalbumin, and bovine serum albumin.

MATERIALS AND METHODS

Ferritin, ovalbumin, rabbit antiferritin and anti-BSA were from Sigma Chem. Corp. Rabbit antiovalbumin was a gift from Dr. David Govol.

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Biotinyl-*N*-hydroxysuccinimide ester (BNHS) was prepared and used to label the antibodies as described previously (7). The antibodies were coupled to Sepharose by the cyanogen bromide method as described by Wilchek et al. (8). ^{125}I-labeled avidin was prepared as previously described (9). For RIA tests, various dilutions of antigen were incubated overnight at room temperature with the corresponding antibodies immobilized on Sepharose. The beads were washed and incubated with biotinylated immunoglobulins (5 μg/ml) for 2 h at 37°C. The beads were washed and incubated again with ^{125}I-labeled avidin for 2 h at 37°C. They were again washed and counted in a Packard γ-counter.

RESULTS AND DISCUSSION

The quantitation of BSA in which biotin labeled immunoglobulin was used is shown in Fig. 1. The sensitivity limit we obtained was only about 2 ng/ml, and this is due to the low specific activity, 10^5 cpm/μg, of the ^{125}I-labeled avidin we used. Similar sensitivities were obtained with ferritin and ovalbumin. Much higher sensitivity can be obtained if ^{125}I-avidin with higher specific activity is used, but higher incorporation of iodine by the direct iodination is accompanied by loss of biological activity. We are now in the process of preparing ^{125}I-avidin with high specific activity, using Bolton–Hunter reagent, or by the use of polytyrosyl-avidin followed by direct iodination. The sensitivity obtained is comparable to the direct RIA in which the corresponding purified ^{125}I-labeled antibodies were used. We have also used the avidin-biotin complex for the development of solid-phase enzyme immunoassays. The many possible uses of the avidin-biotin system in enzyme immunoassays is described in a recent excellent study by Guesdon et al. (10).

In summary, the possibility of using the avidin-biotin system in immunoassay and receptor assay may widen further the application of the avidin-biotin complex in biology.

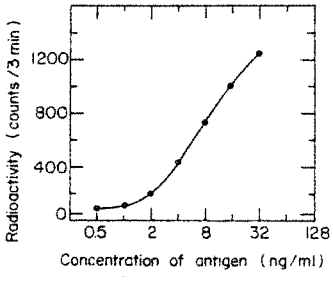

FIG. 1. Quantitation of BSA, using biotinylated antibodies. Sepharose-bound antibodies were successively incubated with their corresponding antigens, biotin-labeled antibodies, and finally with ^{125}I-avidin.

REFERENCES

1. BAYER, E. A., and WILCHEK, M. (1978) Trends Biochem. Sci. 3 : N257–259.
2. BAYER, E. A., and WILCHEK, M. (1980) Method Biochem. Anal. 26 : 1–45.
3. HIEZMANN, H., and RICHARDS, F. M. (1974) Proc. Natl. Acad. Sci. USA 71 : 3537–3541.
4. BAYER, E. A., SKUTELSKY, E., WYNNE, D., and WILCHEK, M. (1976) J. Histochem. Cytochem. 24 : 933–939.
5. BAYER, E. A., WILCHEK, M., and SKUTELSKY, E. (1976) FEBS Lett. 68 : 240–244.
6. ANGERER, L., DAVIDSON, N., MURPHY, W., LYNCH, D., and ATTARDI, G. (1976) Cell 9 : 81–90.
7. BECKER, J. M., and WILCHEK, M. (1972) Biochim. Biophys. Acta. 264 : 165–170.
8. WILCHEK, M., BOCCHINI, V., BECKER, M., and GIVOL, D. (1971) Biochemistry 10 : 2828–2834.
9. WYNNE, D., WILCHEK, M., and NOVOGROSSKY, A. (1976) Biochem. Biophys. Res. Commun. 68 : 730–739.
10. GUESDON, J. L., TERNYNCK, T., and AVRAMEAS, S. (1979) J. Histochem. Cytochem. 27 : 1131–1139.