Methods in ENZYMOLOGY

Volume 184

Avidin-Biotin Technology

Meir Wilchek
Edward A. Bayer

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AVIDIN–BIOTIN TECHNOLOGY

METHODS IN ENZYMOLOGY

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Preface

The development of avidin–biotin technology has unified and simplified a variety of technologies in which antibodies, lectins, hormones, and other binders have been used to localize, isolate, and identify the corresponding antigen, sugar moiety, receptor, etc. In all of these studies, in order to detect the latter, the binder counterpart had to be modified in some manner with a specific marker. In many cases, the preparation of protein–protein conjugates were required. The use of such conjugates resulted in a number of problems, often due to the modified physical and chemical state of the binder, which could lead to an alteration in the specificity and activity of the resultant conjugate.

The contribution of the avidin–biotin system is manifold. Foremost, of course, is the remarkable affinity between the two biomolecules. An added dimension of the system is the presence on egg white avidin (or its bacterial relative, streptavidin) of four biotin-binding sites which often generate an amplified signal. Moreover, avidin and streptavidin are exceptionally stable proteins, amenable to a wide variety of modifications and conjugations which only negligibly disturb the activity or specificity of their biotin-binding properties. Thus, protein–protein conjugates between the binder and marker are unnecessary, and only the biotinylation of the binder is required. Subsequent interactions are accomplished using the appropriate avidin- or streptavidin-associated probe.

In organizing this volume, we would have been delighted to have been able to present definitive studies which could be used as a basis for the design of new systems and applications. Unfortunately, this was not possible. In this explosive phase of the development of avidin–biotin technology for different applications, we chose to present the currently established uses from which (combined perhaps with a touch of imagination) the experienced investigator should be able to extrapolate information for the design of new and exciting uses for this complex.

There is much repetition, particularly with respect to the biotinylation procedures and the preparation of avidin-associated probes. Since the former is critical to most applications, we could not check the validity of each procedure, but have provided a general detailed chapter describing protocols and guidelines for the biotinylation of proteins based on our own research. A definitive opinion on whether a spacer group is required between the biotin moiety and the binder or probe is not given. In some cases the spacer group is definitely required and an enhanced or stabilized signal is received, but in others there appears to be no advantage to its

use. Again, the properties of each system must be determined empirically. A clear stand has not been taken on whether an avidin-conjugated probe should be used or whether a two-step approach (using either sequential treatment or preformed complexes of native avidin and biotinylated probe) should be employed. Again, each researcher must determine experimentally which approach is most pertinent for the project involved.

We would like to acknowledge Mrs. Dvorah Ochert for her indispensable assistance with the manuscripts, the correspondence, the editing, typing, and retyping, all this in addition to her heavy departmental responsibilities. We also would like to thank our wives and families for their patience and understanding during the periods of heightened activity. And, of course, we are grateful to all of the contributors for their cooperation in preparing their manuscripts for this volume.

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EDWARD A. BAYER

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