Chemical Modification of Proteins

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CHEMICAL MODIFICATON OF PROTEINS

preface

Protein chemists have long been interested in altering the chemical, physical, and biological properties of proteins by chemically changing their structure. One of the first things discovered about proteins was how easily they were changed upon treatment with chemical reagents. Their lability to chemical reagents and reaction conditions has been a serious problem for many purposes. The application of modern knowledge of proteins, new chemical reagents, and more sophisticated analytical techniques, however, has made chemical modification of protein molecules one of the most useful approaches to the study of many of their properties.

One of the most frequent questions asked about biologically active proteins is, "What is unique about the structure that accounts for the particular activity?" Thus, interest in most enzymes is primarily in the "active center," the amino acid side-chain groups of the protein molecule that participate in the activity. The objective of this book is to describe chemical methods used to determine the roles of the individual amino acid side chains in the chemical, physical, and biological properties of proteins. Emphasis is given to the use of these procedures for the identification of side-chain groups participating in the "active center."

There are two main parts of this book plus an appendix. The first part, some 65 pages, is devoted to general discussion of the subject and related topics of importance. The second part, including 145 pages divided into five chapters, describes the reagents used for the nondestructive chemical modification of proteins. Subsections are arranged according to the reagents, with similar or related reagents being grouped to make chapters. Each reagent is described in detail as to its properties and use and the properties of derivatives likely to be formed upon its reaction with a typical protein. Expected changes in properties, side reactions, and analytical considerations needed to characterize the modified proteins are included. Examples are given in most sections illustrating the use of each reagent. The appendix contains experimental

procedures taken from the original literature and is intended to enable the reader to perform some of the more important modification reactions.

The book has been developed from the material used for a two-credit one-quarter graduate course intended for students who have had a prior knowledge of general protein chemistry. The course was designed to provide a student with a fundamental knowledge of the chemical modification of proteins with the hope that this knowledge would enable him to select, and even develop, those chemical reactions most suited to his research objectives.

This book would not have been possible without help and advice from many sources. Special appreciation is due to R. D. Cole for a critical appraisal of the initial draft. We would also like to thank the many other friends and colleagues who helped in preparing this material. These include J. R. Whitaker, F. Wold, M. Friedman, F. H. Carpenter, N. E. Gentner, F. C. Greene, S. Govons, Ahmed Ahmed, J. Vandenheede, Ruth Uy, C. Ho, D. Osuga, W. Benisek, G. Blankenhorn, and Y. Lin for reviewing parts of the manuscript and offering helpful criticisms. We also express our appreciation to Judy Miller, Kathy Else, Judy Tweedie, Carolyn Brumley, Sue Brown, and the publisher's staff, especially Sally Anderson, for their valuable assistance in literature searchings and in editing and proofreading the manuscript.

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contents

I

the chemistry and chemical reactions of proteins

1

proteins and their properties

Proteins are extremely complex molecules. This is illustrated by the model of a relatively simple protein, chicken egg white lysozyme, shown in Figure 1-1. Its complexity is due to its relatively great size and to the unique three-dimensional arrangement of its many functional groups. The fascinating physical, chemical, and biological properties of these indispensible components of living matter are subjects of great interest. Relating their various properties to specific structural features is the basic goal of protein chemistry.

1-1 HISTORICAL DEVELOPMENTS

Early History. The early history of protein chemistry was primarily concerned with the chemistry of the individual amino acids and with the synthesis and properties of small peptides. Fischer's work on amino acids and peptides, including some of the first chemical syntheses of peptides, is one of the outstanding classical stories of organic chemistry. Sumner's demonstration in the 1920's that enzymes are proteins stimulated interest toward identifying particular amino acids responsible for their catalytic activity. Many of the chemical methods for proteins developed during this period, however, were for quantitatively determining the amounts of individual amino acids in proteins, rather than for modifying them.

A great acceleration in the development of chemical methods for the study of proteins occurred during and immediately following World War II. Important developments of this period were the reviews of protein modification written in 1947 (Olcott and Fraenkel-Conrat, 1947; Herriott, 1947). Some of the procedures described in these publications are still very much in use. An important landmark a few years later was the demonstration by Balls and Jansen (1952) that the specific

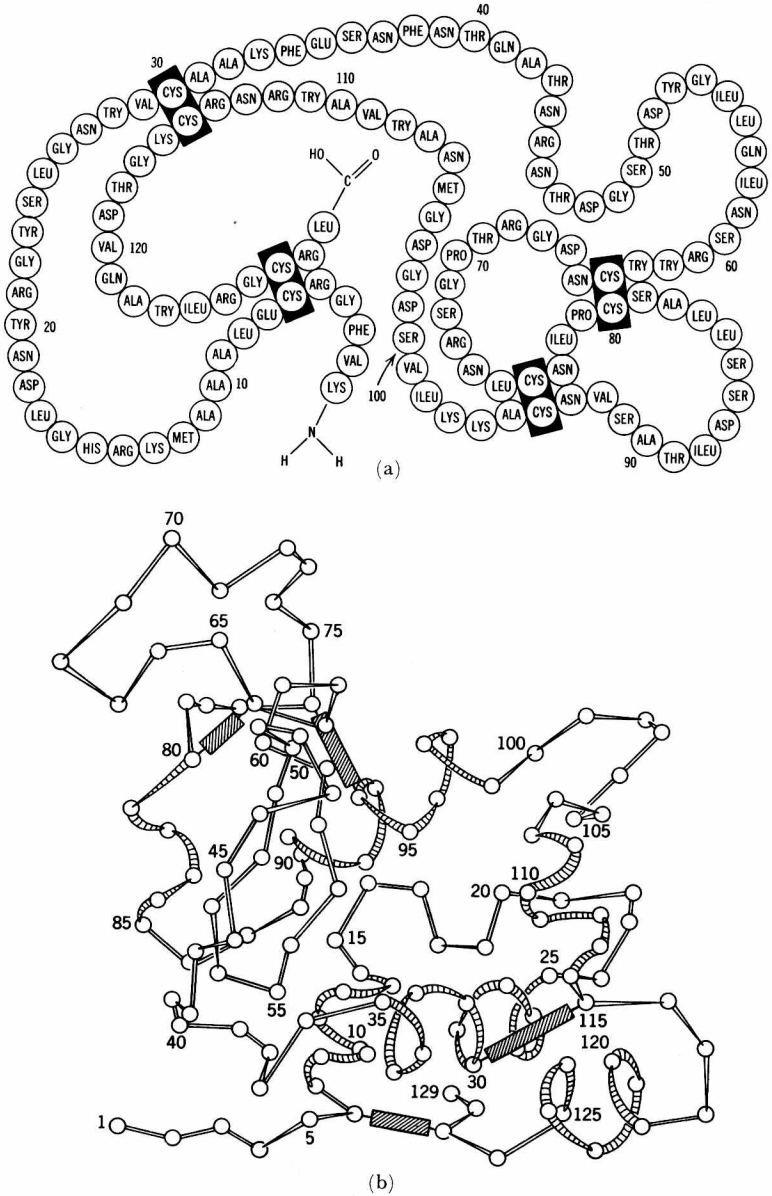

FIGURE 1-1 (a) The structure of egg white lysozyme indicating the positions of the four disulfide bonds. ASN and GLN denote asparagine and glutamine, respectively. (From Canfield and Liu, 1965.) (b) Schematic drawing of the main chain conformation of lysozyme. (By W. L. Bragg, from Blake et al., 1965.)

inactivation of certain proteases by diisopropylfluorophosphate involved reaction with specific serine residues presumably in their active sites.

Scientific developments during the following years led to increased interest in several physical-chemical techniques for studying proteins which, in turn, stimulated interest in protein modification. The use of X-ray diffraction for the study of proteins, for example, promoted interest in methods for isomorphic introduction of heavy atoms into proteins (see Section 3-2). For other studies, techniques for enhancing the fluorescence of proteins by introducing various fluorescent groups were developed. Improvements in many physical-chemical techniques have both contributed to, and made use of, protein-modification techniques.

The development of more accurate and more sensitive analytical techniques has similarly been a major contributor to, and beneficiary of, advances in protein modification. Development of the automated amino acid analyzer has been one of the most important improvements, having contributed immeasurably to all of protein chemistry.

Recently, great interest has been directed to the search for "affinity-labeling" or "active-site-directed" reagents. These reagents are designed to react preferentially with only those parts of protein molecules that are physically in the vicinity of particular biochemically active sites. Their use gives a highly selective way of chemically modifying a protein (see Section 2-2).

1-2 CHEMICAL MODIFICATIONS FOR ANALYTICAL AND INDUSTRIAL PURPOSES

For many years a motivating factor in the study of the chemical modification of proteins was the desire to determine quantitatively the amounts of proteins or their component amino acids. Many methods have been developed for such purposes. Because the intent was not to preserve the integrity of the protein, many of these methods are very harsh and in this way differ from most procedures described in this book. A few methods originally designed strictly for quantitative purposes have, however, also proved useful for selective chemical modification of proteins. The use of nitrous acid for the determination of amino groups in proteins, for example, is also of value for their selective modification.

Only a few methods are suitable for both modifying and determining amino acid side chains in proteins. Two such methods now in common usage are the reaction of sulfhydryl groups with *p*-mercuribenzoate and the reaction of amino groups with trinitrobenzenesulfonic acid. Both reagents can be used under relatively mild conditions which do not damage most proteins, and it is easy to measure the numbers of groups modified.

Commercial applications of the chemical modification of proteins have a long history related to the pharmaceutical, dyeing, and clothing industries. An early

application in the pharmaceutical industry was the use of formaldehyde to modify bacterial toxins and viruses; similar procedures are still important commercially. The purpose of this treatment is to kill, inactivate, or so change the virus or toxin as to render it incapable of eliciting its toxic or pathological response, while retaining its ability to elicit an immunogenic response when injected into an animal. The bacterial toxins, when so modified, are known as *toxoids*.

One of the oldest processes involving protein modification is the treatment of animal hides or hair for human use, as in the tanning of leather. Increased knowledge has led to recent improvements in these ancient procedures. For example, glutaraldehyde, used for cross-linking of proteins, is now also used for tanning leather. It apparently functions similarly by cross-linking collagen in the leather. Similarly, several different modifications are now used to give wool fibers superior performance for clothing. Chlorination or treatment with polyepoxides is being used commercially. The latter primarily react with amino groups. Use has also been made of reagents splitting disulfide bonds for the purpose of obtaining "permanent press" in finished clothing items.

1-3 CURRENT STATUS

The current interest in chemical modifications of proteins is indicated by the many reviews and books on its various aspects. Baker (1967), for example, has published a book dedicated to the organic chemistry of the active site (of enzymes), and Hirs (1967) has edited a detailed compilation of laboratory procedures for proteins. Other reviews, including general discussions of chemical reagents and their reactions, have been published by Cohen (1968), Glazer (1970), Vallee and Riordan (1969), Stark (1970), Shaw (1970), and Spande et al. (1970).

There is now a long series of chemical reagents and reactions used for: (a) synthesizing peptides and proteins (Stewart and Young, 1969), (b) sequential and stepwise degradation of proteins to determine their structures (Stark, 1970), and (c) preparation of derivatives of amino acids to increase their volatilities and detectabilities by vapor phase chromatography (Gehrke et al., 1968). The first type under (a) includes methods which must maintain the integrity of the polypeptide chain and the structures of the side groups of the amino acids, because the objective is to end with a normal polypeptide or protein. Special reagents are used to block or stabilize the partially synthesized protein. In general, however, the methods are milder than (b) and (c) types. This is not the case for the methods used for preparing derivatives for vapor phase chromatography. For this purpose, the only requirement is maintenance of the structure of the individual amino acids. Consequently, the methods used for these derivatives usually involve much harsher conditions than are used for intact proteins.

Many of the methods in use today are sufficiently simple and convenient to be