Volume 6

CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS

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Boris Weinstein

CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS

A Survey of Recent Developments

◄ *Volume 6* ►

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ABOUT THE SERIES

The amide bond is one of the less reactive organic functional groups, yet it serves as the cornerstone for the building of the many peptides and proteins found in living systems. The evolving science of molecular biology has served to stress again that the chemistry and biochemistry of amino acids, peptides, and proteins is interwoven into a complex pattern, which on closer examination is found to be dependent on a host of secondary factors associated with individual compounds. There has been a need for a new review series in this area, especially if the interrelationships between the various disciplines are to be discussed in a detailed fashion. In an ideal sense, each volume should contain some chapters on recent developments and applications of established techniques, whereas others might describe the background and problems for topics still under investigation. Too, the subjects encompassed here do permit a variety of treatments without undue duplication or specialization.

One need not remind the reader of the many life processes that are dependent upon specific amino acid, hormone, and enzyme systems. Each functions in a very unique fashion, yet, in the end, they must involve the reactions of fundamental organic chemistry. Sometimes this point is overlooked and it will be restated in greater detail through the series. To balance the scale, the brief comment is made here that new protecting, labeling, and coupling agents are always desirable, but these must be put to the test by the synthesis or degradation of actual compounds, for which practical use exists in Nature.

It is anticipated that these volumes can be useful both to the specialist and nonspecialist and may provide a reference point to those who may do research in a broad region, or to the active worker

in a small field. Most importantly, these volumes can serve the general purpose of presenting various points of view on the amide bond to interested observers, who, at present, are unknown to one another.

Seattle, Washington BORIS WEINSTEIN

PREFACE

Ten years ago the first volume in this series emerged and it is of interest now to consider the many changes seen in peptide research as mirrored by the various contributors to the six books that bear the common title of *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*. Some thirty-eight authors have generated thirty chapters to date and it is a pleasure to thank them again, both individually and as a group, for their assistance and help. To be sure, the choice of a certain topic (much less getting a desired manuscript by a specific deadline!) is a gamble, yet the act of putting together these books is an interesting story and one that hopefully shall continue for a long time. An appeal is again made to those who are interested in peptides to suggest potential subjects for review. An esoteric technique or development may be of wide interest and those who work on peptides always need to remain alert to better methods or to learn about advancements in related fields.

To move to this specific book, the first chapter by Walter Hill discusses ribosomal proteins, while Charles Stammer returns as an author with a contribution on dehydroamino acids and proteins. Peptides that complex transition metals are next considered by Loren Pickart, while Ettore Benedetti has prepared a summary of recent activities in X-ray crystallography that bear on the structure and conformation of peptides. Scott Chilton has written an interesting account of secondary amino acids found in mushrooms, while Ivar Ugi, Dieter Marquarding, and Reinhard Urban review the synthetic applications of four-component condensation. In summary, the reader can look forward again to a delectable treat centered around the amide bond.

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CHAPTER 1

RIBOSOMAL PROTEIN SYNTHESIS AND STRUCTURE

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I. INTRODUCTION

The purpose of this chapter is to provide an overview of the results of studies on ribosomal proteins, their synthesis, and structure. It is impossible for this to be done without also discussing the ribosomes themselves and the structure and function of the entire ribosomal structure. This, in turn, calls for an in-depth discussion

of protein biosynthesis, which is much beyond the purview of this survey.

Therefore, this chapter will limit itself to a summary of the ribosomal proteins, their synthesis and structure, and the topographical placement of these proteins on the ribosomal subunits. No effort will be made to discuss any details of the function of the individual proteins or groups of proteins, for so doing would necessitate the incursion into the realm of protein synthesis.

Most of this discussion will center on studies of ribosomal proteins derived from bacteria, notably *Escherichia coli*. Of necessity, this survey will not be complete and regrets are expressed to the many authors whose work has been omitted. The literature is covered to the end of 1978.

II. BACKGROUND

There have been several reviews [2,3,6,7,10,11,14-16] and a book [12] published about ribosomes in recent years. The historical development of ribosomal studies is especially well covered in the latter reference. For ribosomal proteins, the field is yet moving so fast that there does not exist a complete overview. However, several articles do discuss some aspects of ribosomal proteins rather extensively [4,12,15].

Initial studies in the 1960s clearly showed that ribosomes were like viruses in that they were ribonucleoproteins, yet unlike viruses had a great number of different proteins [55,94,163,164]. It was also shown that the proteins did not encapsulate the RNA strand, but rather were mixed with it.

By the turn of this past decade it was shown that ribosomal proteins were not only heterogeneous, but that they were unique, except for one duplicated protein (L7/L12*) on the 50S subunit and a duplica-

*S1-S21 designates proteins found on the 30S subunit. L1-L34 designates proteins found on the 50S subunit. DNA, mRNA, RNA, and tRNA have their usual meanings.

tion of one protein between the two subunits (S20/L26). One of the perplexing questions asked quite early was what constituted a ribosoma protein [89,165].

Various preparative methods, including high-salt washes and precipitation, were used to isolate the "pure" subunits [77]. Simultaneously, it was shown that these washed ribosomal subunits (especially the 30S subunit) were heterogeneous with respect to protein content [90,155]. For some time the evidence supported the idea that there may be several kinds of ribosomal subunits, each having its own particular complement of proteins. However, further study [74] showed that all ribosomal proteins were present as one copy per ribosome except for multiple copies of protein L7/L12 on the 50S subunit. It was subsequently shown that the preparative techniques were removing proteins. Even yet techniques have not been developed to homogeneously isolate ribosomal subunits containing a full complement of proteins.

Another problem also complicated the isolation of ribosomal proteins. Many protein factors (e.g., initiation, elongation, and termination) are needed for protein biosynthesis to take place. These proteins are often bound to the ribosome-mRNA-tRNA complex and as such are isolated with the ribosomes and ribosomal proteins. Methods have now been developed to isolate, purify, and characterize most of these factors.

Early methods of isolating ribosomal proteins consisted of treating the ribosomal subunits with varying high concentrations of different salts that cause a release of groups of proteins [143,145]. Another method was developed [75] wherein the proteins in a lithium chloride (LiCl)-urea buffer were eluted from a phosphocellulose column using an increasing salt gradient. This procedure made it possible to isolate many of the 30S proteins homogeneously. Additional techniques, such as reeluting some fractions from the phosphocellulose column through G-100 Sephadex [81,121], were used to isolate the remaining heterogeneous elution products. More recently a method has been developed

which allows the separation and purification of the ribosomal proteins without denaturing them [101].

In order to characterize the purity of the individual proteins, polyacrylamide gel electrophoresis was used to separate the proteins. Initially this consisted of using disc gels of varying hardness to separate the proteins [162]. However, since most of the proteins were basic and several had virtually identical mobilities, it was impossible to identify all proteins present in a preparation. Therefore, a two-dimensional (2-D) gel electrophoresis technique was developed [86] that has now been refined and miniaturized to conserve sample without loss of resolution [98]. Using these 2-D techniques, it was possible to identify 21 different 30S proteins and 34 different 50S proteins. These have each been numbered S1 to S21 and L1 to L34, respectively, from positions noted on the 2-D gels.

There are still some ambiguities present. For instance, protein L7/L12 is always present as a dimer. Proteins L7 and L12 are identical except that protein L7 is acetylated at the N-terminal end [152]. Proteins S20 and L26 have also been found to be identical [168]. Evidence has also come forth recently that L8 is really a complex of L7/L12 and L10 [128]. A very recent report has provided evidence that S1 in *E. coli* really comes in two distinct forms, S1 and S1A, having a difference in molecular weights of about 4000 daltons [161]. Therefore, the absolute number of unique ribosomal proteins is still open to question.

It is now possible, given suitably large amounts of ribosomal subunits, to prepare quite homogeneous fractions of almost all of the 54 ribosomal proteins. This step is essential to further characterization of the proteins.

Antibodies to each of the 54 ribosomal proteins have also been made [149] and using these, some exquisite studies have been carried out which will be described in the following sections.