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Solid Phase Peptide Synthesis

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SOLID PHASE PEPTIDE SYNTHESIS

Solid phase peptide synthesis is a relatively new biochemical process for polymerization of amino acids. It involves the selection of amino acids, blocking groups, and resins. This procedure was first investigated by R. B. Merrifield in an attempt to simplify previous methods of peptide synthesis.[1] Since other methods involved α-amino acids, peptide esters, and diketopiperazines, peptide synthesis had been limited by amino acid selection and a host of technical problems.[2] Solid phase synthesis alleviates many of these difficulties and limitations by supplying a faster and simpler stepwise addition of a wide variety of naturally occuring and synthetically prepared amino acids to a solid resin support. The new process has opened new areas of biosynthesis as more study is made concerning proteins and polypeptides.

The peptide bonds which link together the protein or polypeptide are formed in solid phase synthesis by the interaction of the amino groups with carboxyl groups of the amino acids.[3] The specificity with which these linkages are located is due to the position of the amino and carboxyl groups on the amino acid and the placement of the blocking groups on the functional groups. By careful selection of each amino acid, the construction of biochemically active proteins, enzymes,

and polymers may be effected. To enable synthesis of these active substances a study of the properties of the amino acids, the composition of the desired product, and its action should be made.

Vital to the synthesis of a polypeptide is the selection of protecting groups. The purpose of protecting groups is to render the specified functional groups and side groups on the amino acid inactive in order to modify the polypeptide or amino acid and effect bond formation. Protecting groups should be selected in a manner such that it is easily attached to the amino acid and removed from the peptide at the proper time without modification of the peptide. There are many such protecting groups in wide usage today. Blocking groups which are attached specifically to the amino group form acid derivatives. Definite problems arise due to the basicity of the amino groups.[4] Thus selection of protecting groups is limited when concerned with peptide synthesis. This restriction can be eliminated at high pH values at which the amino group becomes a nucleophile and therefore increases its reactivity with most reagents.[5] Attachment of protecting groups to the amino group on the peptide or amino acid is accomplished by esterification. The carboxyl groups can be effectively protected in many ways. Salts of the amino acid can be formed as blocking groups, but as a result these tend to be soluble in water, thus aqueous solvents employed in these reactions create technical difficulties in peptide synthesis.[6] As alternatives ester formation, saponification, and acyl derivatives are used as protecting groups. In these cases the resulting derivatives render the amino group soluble in organic solvents which in the synthesis of peptides are preferred. In recent years esterification of the carb-

oxyl groups have been used to a wider extent on account of the ease with which the protecting group is removed by acidic hydrolysis. During saponification reactions, subsequent side reactions may take place, consequently modifications in the peptide chain may result from these side reactions. Many similarities concerning the protection of side groups such as hydroxyl groups, thiol groups, and carboxyl groups can be noticed since each have similar characteristic functions. Side chain carboxyl groups react much like terminal carboxyl groups and can be protected in the same manner, that is by esterification. It should be noted that with some ester formations a rearrangement may take place upon hydrolysis. Care should be taken in the selection of these derivatives in order that no undesirable modifications are made within the peptide chain. Hydroxyl groups should be protected so that during synthesis, ester formation involving the hydroxyl group does not take place. These side groups can effectively be protected by converting them to ethers.

Removal of these protecting groups is accomplished by acidolysis or hydrolysis of the ether.[7] When the synthesis of a peptide involves an amino acid with a thiol side group, it is mandatory that this group be protected. Since the thiol is more acidic than the hydroxyl group, its nucleophylicity is greater than that of the hydroxyl group and subsequent side reactions may take place. Thiol groups may be protected by a conversion to thioethers which may be cleaved under acidic conditions.

Examples of these protectors for amino groups, carboxyl groups, and side chain groups are t-butoxycarbonyl azide, n-benzyloxycarbonyl azide, p-nitro-benzyl esters, t-butyl ethers and t-butyl thioethers,

respectively.

In solid phase peptide synthesis, the terminal carboxyl group is attached to an insoluble solid support called a resin. These resins are polymeric in nature and are usually beads of 200 to 400 mesh of polystyrene or a related compound. The carboxyl group is attached to the resin by an esterification reaction and following the peptide synthesis is removed by saponification. In certain cases the resin may be nitrated or brominated to increase the yield of the product. As a result of vigorous conditions which usually accompany the removal of the peptide from its resin, cleavage of other bonds or chain modifications may occur. These substituents on the resin possess deactivating properities and allow the separation of the peptide from its resin to proceed under milder conditions. Careful selection of blocking groups which are not modified under these harsh conditions will not require specified resins. Varieties of resins have been used in experimentation, but the partially chloromethylated polystyrene resin seems to be favored over other resins. The reason for this may lie in the practicality and ease with which the carboxyl group may be attached by t-butyl ester formation. The use of a resin is responsible for the simplicity and ease characteristic to solid phase peptide synthesis.

The formation of a polypeptide is carried out in a specific stepwise manner. After the selection of amino acids for the preparation has been made, attachment of necessary blocking groups to terminal function groups and active side chain groups is the first step in the synthesis. It may be profitable to attach blocking groups to each amino acid before continuing with the synthesis. However accomplished, it is mandatory that each amino acid have a blocking group on either

the amino group or carboxyl group and any side group prior to any other step in the procedure. If this is not done, consequent side reactions or premature linkage may occur. Following blocking group attachment is the esterification of the carboxyl group to the resin. After the formation of the protected amino acid resin derivative is completed, the removal of the protecting group from this derivative is the next step in the sequence. This prepares the derivative for peptide bond formation with the next n-terminal protected amino acid by condensation of the free carboxyl group with the amine group attached to the resin. The same process is carried out for each subsequent addition of a protected amino acid. After the desired composition of the polypeptide has been achieved, removal of the protecting group on the end amino acid is effected. Following this step is the removal of the polypeptide from its resin. Since the polypeptide is in a convenient form, it is advisable to purify it from excess materials before the final separation of the polypeptide and resin is made.

Purification of the polypeptide includes distillations and extractions of solvents used in protecting group and resin attachment, and washings necessary at different phases of the synthesis. Depending upon the solvent, distillations may be simple, fractional, or carried out under diminished pressure. Extractions, like distillations, vary because of chemical composition in each stage. The type solvent to be used is selected due to the position of the material to be extracted. These materials may be found in aqueous or nonaqueous phases. It is sometimes necessary to change the composition of the nonaqueous phases or change the pH of the aqueous phase before extraction can be carried out. Solvents used for washings should be selected in a manner such that in the removal of undesired materials they do not result

in modifications of the polypeptide desired. Upon completion of purification procedures, the final removal from the resin can be made and the polypeptide will be ready for tests in determination of structure.

Like all other aspects of peptide synthesis, there is a variety of methods for analysis of polypeptide chains. The most frequently used method is terminal residue analysis originated by Frederick Sanger in 1945 at Cambridge University.[8] This procedure involves the formation of derivatives of either the terminal amino or carboxyl group on the polypeptide, followed by hydrolysis of the peptide linkage. This test is especially well suited for analysis of long chain polypeptides. The hydrolysis which is used to break the peptide linkage is mild enough to leave the rest of the peptide intact and analysis of the new terminal group of the shortened peptide group can be made.[9]

The purpose in synthesis of polypeptide is that through these procedures more information of biochemical activity can be obtained. The knowledge gained from experimentation in the area of polypeptide and protein biosynthesis may be applied to medicinal and industrial usage. However, before practical application of biosynthesis can be made, the biological activity, due to conformations, functional groups, and the specificity with which they act, should be considered.

Activity resulting from the conformational differences in peptides and proteins can be duplicated by careful choices in amino acids, and the sequence in which they are linked. The biological activity of natural polypeptides is closely related to its structure. Most polypeptides contain folded chains. Denaturization is the destruction of these folded chains and the resultant protein becomes inactive and insoluble. The folding which occurs in globular proteins is

highly complex. To achieve the folding in the synthesis of a polypeptide, the attachment of side groups capable of intramolecular bonding should be utilized. These bonding forces may be Van der Waals forces, hydrogen bonding, or a condensation for a more permanent attachment. By reason of specified synthesis, the structure of naturally occurring folded polypeptides is becoming universally recognized. In natural peptides the folding may be at random, incomplete, or lacking. This mixture of folded types may cause definite action of the part of the polypeptide. Therefore, when synthesis of this peptide has been completed, the new peptide will not possess this mixed quality. By comparison of the synthetic with the natural peptides, activity can be identified with the presence or absence of certain components of this natural mixture.

Two neucleic acids, ribose and deoxyribose, are responsible for naturally occuring synthesis of polypeptides and proteins. As investigation of folded chain characteristics continues, knowledge of these two important globular polypeptides will increase. Natural synthesis is carried on within the cells of plants and animals under the direction of the master blueprint DNA. The macramolecule , DNA, serves as a template for the synthesis. The template consists of an order of purine and pyrimidine bases which are specific for each amino acid in the polypeptide synthesis. Since DNA is found in the nucleus of the cell, this template is duplicated by the RNA found within the nucleus. This RNA, called messenger RNA, (mRNA), matches purine and pyrimidine base groups on the template with its own bases and carries them to the ribosomes in the cytoplasm. At this point another type of RNA, ribosomal RNA, (rRNA), whose function has not yet been determined, takes over.

It is felt that RNA provides proper spacing and orientation for mRNA. Following the orientation of the template on the ribosome, is the transfer and allignment of amino acids on the template. This is done by transfer RNA, (tRNA). Since each amino acid is specific in its orientation, the same polypeptide or protein is replicated in this manner. After the amino acids have been correctly aligned, the final step is the enzyme-catalyzed formation of the peptide linkages between adjacent amino acids. As this bonding progresses, tRNA is released so that it may continue in the synthesis, but mRNA must be replaced. The precision with which a protein or polypeptide is replicated is postulated in this manner. Because of the complexity involved in structural replication, transfer of materials, and the sequence of events in natural polypeptide and protein synthesis, this general explanation is hardly justified. This natural phenomenon is a complete study in itself. As more investigation is made in this area, perhaps a new method of peptide synthesis will be discovered.

In the synthesis of enzymes, particular care must be taken in selection of active groups. Since enzymes are specific catalysts for biochemical reactions, their synthesis must include the attachment of groups which function as active sites. Their activity depends upon the ability to form an enzyme-substrate complex which is determined by their structure. If these criteria are not met, the action of the enzyme is nullified.

Practical application of biosynthesis may appear in medicine or industry. With the ability to synthesize biologically active enzymes and proteins, new methods for medicinal usage are being hypothesized. An example of these methods is the transformation reaction which DNA

undergoes in a bacterial cell to enable immunity to detrimental substances.[10] Other medicinal uses of biosynthesis can be found in pharmacology. The industrial values of protein and polypeptide biosynthesis is seen in the mechanization of synthesis, production of synthetic fibers, beef tenderizers, dye attachment, and structural modifications for the purpose of obtaining "permanent press" fabrics.[11] With more experimentation, new items for industrial, as well as medicinal usage, will be produced.

The synthesis of the dipeptide glycyl-D-L-serine was accomplished in a manner very similar to the procedure described earlier. Following the selection of amino acids, the choice of t-butoxycarbonyl azide as an n-terminal blocking group was made. This protecting group was quite suited for the dipeptide synthesis since it did not require a nitro or bromine substituent on the polymeric chain of the resin. It was attached by the formation of an ester with the amino group on the amino acid. This was done in dioxane, with magnesium oxide and water.[12] The resin chosen for synthesis was the cloromethylated polystyrene or Merrifield's resin. It was attached to the amino acid D-L-serine by the formation of an ester with the free carboxyl group. This was done by refluxing the protected amino acid in ethylacetate for 24 hours.[13] The peptide bond between the amino acids glycine and serine was made after the amino acids had been protected and the D-L-serine had been attached to the resin. At this point, the blocking group of serine was removed and a condensation reaction was responsible for the formation of the peptide linkage. The new peptide was washed free from impurities with methylene chloride, ethanol, and acetic acid.[14] The specifics concerning the formation of the dipeptide glycyl-D-L-serine can be found in the laboratory manual.[15]

Solid phase peptide synthesis is an orderly sequence of selection and attachment of protecting groups to amino acids and the protected amino acids to resins. Its relatively new discovery has opened new doors of biosynthetic study for both medicine and industry. Because of the ease with which it is accomplished, it provides a faster and simpler way to study new synthetic peptides. As more study is made concerning the biosynthesis of polypeptides and proteins, new methods for synthesis will be discovered.

FOOTNOTES

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