essary for self-sufficiency will certainly prevent the attainment of this goal within the foreseeable future. Still, it remains a goal toward which we are moving.

Meanwhile, we may expect to see the development of a number of self-sufficient regional calibration centers. With such centers in existence, the national standards laboratories may no longer need to make many calibrations; they can then concentrate more on the development and dissemination of procedures that will permit every major laboratory to reproduce accurately the standard for each quantity.

References and Notes

1. Unfortunately the word *standard* has multiple meanings in English. In this article the word is used to mean a standard for physical measurement; that is to say, the physical realization of an agreed-upon unit (such as the meter or kilogram) for the expression of physical measurements.
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16. I wish to acknowledge with gratitude the extensive assistance and participation of Mr. W. K. Gautier in the preparation of this manuscript.

Automated Synthesis of Peptides

Solid-phase peptide synthesis, a simple and rapid synthetic method, has now been automated.

R. B. Merrifield

The science and art of peptide synthesis have flourished remarkably in recent years. Achievements such as the synthesis of insulin (*1*) and several other peptide hormones (*2*) show that the present methodology has been very effective and suggest that it will be extended even further. But in spite of these accomplishments the conventional procedures have certain inherent characteristics which will tend to set practical limits on the size of polypeptides which can be synthesized in reasonable time and with reasonable yield. The standard technique depends on building up long chains by repeated condensations of individual amino acids or on combining a series of small peptides to form a single large one. Such multistep processes are very laborious and require large numbers of separate reactions. For that reason synthetic methods of the greatest simplicity and efficiency are essential.

Following earlier work from this laboratory on the synthesis of peptides by conventional methods (*3*), experiments were begun in June 1959 on the general problem of finding a new approach to the synthesis of this important class of compounds. The primary objective was to devise a simple and rapid method, and the ultimate aim was to so simplify the procedures that they could be performed and controlled automatically. Automation was expected to offer a great advantage for the synthesis of large polypeptides, and it appeared to be the only reasonable hope for the eventual attempts to synthesize proteins. By 1962 it was possible to describe the basic idea for a new approach, to demonstrate its feasibility, and to predict its adaptability to automation (*4*). The process was named "solid-phase peptide synthesis" because all the reactions proceeded in a two-phase system in which the peptide was always present in the insoluble solid phase. The present article is intended to outline the principle behind the method, to describe the development of the method, and to show how it was, in fact, automated and how it has been applied to the synthesis of certain biologically active peptides.

Before discussion of the new approach, perhaps it would be best first to review the general procedures involved in standard peptide synthesis and to point out some of the difficulties and limitations (*5*). In essence the problem is simply to form a series of peptide bonds; that is, to prepare amides derived from the carboxyl group of one α-amino acid and the amino group of the neighboring one. Before this can be done, however, it is necessary to block the other functional groups of both amino acids to prevent them from entering into the coupling reaction. The selection of blocking groups which will provide the necessary protection but which subsequently can be effectively removed without disrupting the peptide bond is a major problem. Another is the activation of the carboxyl group in such a way that it will couple in high yield, without side reactions. Furthermore, it is essential to select conditions which will avoid racemization of the asymmetric centers of the component amino acids. The peptide chemist is now in command of a large arsenal of reagents and methods which enable him to accomplish these objectives. But the successful synthesis of the peptide bonds is only part of the overall task. In the standard methods it is equally important to isolate and purify each intermediate product before it can be used for the next step in the synthesis. Each product must be separated from starting materials, reagents, and by-products which, in many instances, will have very similar properties. This

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often is by far the most difficult part of the synthesis. Ideally, the desired product will crystallize in pure form from the reaction mixture; in practice this is common for small peptides, but uncommon for large ones. For large peptides it becomes necessary to resort to other separation techniques, such as chromatography or countercurrent distribution. For multistep syntheses these additional operations greatly decrease the efficiency of the process. When the synthesis of a polypeptide such as insulin (51 residues) calls for 221 individual steps (*6*), it is clear that any but the simplest and most efficient procedures will require an overwhelming amount of time and effort. Yield, too, is a major concern. Unless each of the many reactions can be made nearly quantitative, the yield of product which can be prepared from reasonable amounts of starting materials will be exceedingly small. Consider, for example, that after 20 sequential reactions, in which a relatively high yield of 80 percent is obtained at each step, the final yield from the first residue will be only 1.2 percent. If the efficiency of the individual steps declines to 70 percent, the yield drops to less than 0.1 percent. To bring the final yield over 50 percent, the separate reactions must average approximately 97 percent, and this is far beyond the range usually attainable.

An alternative route to synthetic polypeptides is the polymerization of *N*-carboxyanhydrides (*7*). This technique has been useful for the preparation of peptides of high molecular weight, but it also has severe limitations. It is not suited to the synthesis of peptides containing several amino acids in a defined sequence, which, of course, is one of the important characteristics of naturally occurring peptides and proteins. Second, even for polymers of a single amino acid, the method cannot give a product of defined size. The chain length can only be stated in terms of a statistical distribution.

General Principle of Solid-Phase Synthesis

Solid-phase peptide synthesis (*8*) was designed to avoid or overcome certain of the problems and difficulties just described. It has much in common with the standard methods, but it differs in certain distinct and important ways. The basic idea underlying the new method is that a peptide chain can be synthesized in a stepwise manner from one end while it is attached by a covalent bond at the other end to an insoluble solid support, and that, after the synthesis of the entire peptide is complete, the peptide chain can be cleaved from the solid particle to which it has been anchored. The plan for solid-phase synthesis can be generalized as shown in Fig. 1. The scheme can be applied in principle to the synthesis of a variety of oligomers or polymers of defined sequence and structure. Specifically, for the synthesis of a polypeptide the first individual unit, A_1, is the amino acid which occupies the terminal position at the carboxyl end of the proposed peptide chain. The amino group is masked with a protecting group, p, and is attached to a solid particle at a reactive site, x. The linkage will be by a stable covalent bond. The protecting group is then selectively removed, exposing a new reactive site which is next coupled with the second protected amino acid, A_2, to give the first peptide bond. The deprotection and coupling steps are repeated alternately until the required peptide is assembled. It should be noted that the growing peptide chain is in the insoluble solid phase throughout the synthesis, and all of the reactions, therefore, are heterogeneous ones in which soluble reagents react with the insoluble chain. This is in marked contrast with conventional methods, where every effort is made to have all reactants in solution for homogeneous reactions. After completion of the stepwide synthesis, the bond holding the product to the solid is finally cleaved. The synthetic polymer, now in solution, is separated from the residual supporting particle and purified by standard procedures.

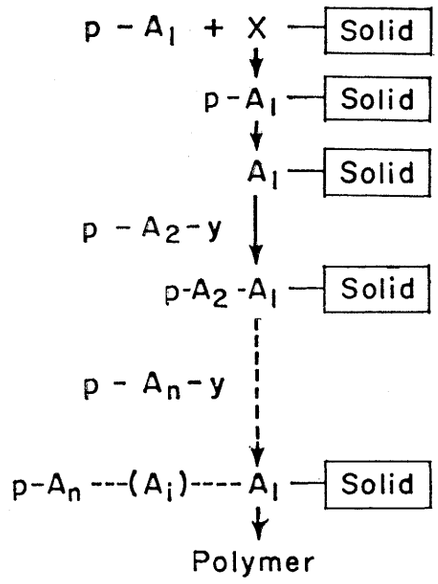


Fig. 1. The general scheme for solid-phase synthesis. A_1----A_n represents the different monomer units which are arranged in defined sequence in the polymer; *p* is a protecting group; *x* and *y* are activating groups.

The general scheme of Fig. 1 is suitable for the synthesis of other classes of polymers. The details would be different, but the approach would be similar. Thus, in the case of a polynucleotide the individual unit would be a protected mononucleotide, for a polysaccharide it would be a suitable protected monosaccharide, and so on.

There are, in theory, several advantages to the new scheme, namely, high yield, simplicity of operation, and speed, and these benefits have been realized in practical applications to the synthesis of certain peptides. The basis for the various advantages lies in the fact that the peptide, when firmly bound to an insoluble solid support, becomes itself totally insoluble and, with proper selection of the supporting material, it becomes possible to filter and wash the solid very efficiently. It can be exhaustively washed to remove soluble, or even only slightly soluble, reagents, by-products, and excess reactants without danger of losses of the desired peptide. Thus, as in any synthesis, some effective purification step is necessary, and in this instance the step is simple washing. The elimination of the classical purification procedures greatly simplifies the overall synthesis and leads to an improved yield by eliminating loss due to mechanical manipulations, as well as to solubility. In addition, the coupling reaction itself can be forced to completion with virtually 100-percent yield by using an excess of the soluble amino acid derivative. Overall yields of purified peptides are usually between 50 and 80 percent. A further advantage is the large saving in time and effort, especially during the intermediate purification steps. This was dramatically demonstrated by the automated system where all the steps were carried out mechanically without tedious manual operations. It was then possible to extend the peptide chain continuously at the rate of six amino acid residues per day.

Since the introduction of solid-phase peptide synthesis, there have been several other experimental attacks on this

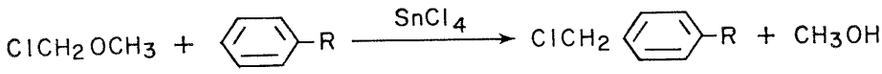


Fig. 2. The chloromethylation step.

general problem of finding new approaches to peptide synthesis. They are related in purpose and bear certain similarities in principle to the one under discussion here. Letsinger and Kornet (*9*) proposed a synthesis in which the first amino acid was attached through its amino group to a solid support, followed by lengthening of the chain by activation of the carboxyl end. Although a dipeptide was synthesized in this way, the danger of racemization might limit the usefulness of this procedure for longer peptides. The synthesis of peptides in solution, but without isolation of intermediates, was carried out in two laboratories (*10, 11*) in efforts to simplify the process. In both methods water-soluble carbodiimides were used for activation; either ion-exchange columns or liquid-liquid extraction procedures were then used for the intermediate purification steps. The overall operations required by these procedures still appear to be too complicated for adaptation to an automated process, however.

Development of the Method

With this general discussion of the principles involved and of the advantages of the new method, a more detailed description of the individual steps involved in solid-phase peptide synthesis can now be given.

The choice of the solid support to be used in the synthesis was of utmost importance. First, the support had to be completely insoluble in all solvents used, which included acidic, neutral, and basic organic solvents, as well as strong anhydrous mineral acid. The material also had to be physically and chemically stable under these conditions. Finally, it had to be in a form which permitted ready filtration. After considerable experimentation, a copolymer made from 98 percent styrene and 2 percent divinylbenzene and in the form of small spherical beads was selected as most suitable (*12*). The low cross-linking was sufficient to impart complete insolubility and good physical stability, and still allowed a high degree of swelling in nonpolar solvents. For example, in methylene chloride the resin expanded to 3.3 times its volume in water or methanol. The opened gel network was then readily penetrated by soluble reagents. It should be pointed out that only a small fraction of the peptide chains were confined to the surface of the beads. Most of the synthesis occurred within the matrix of the resin, and for that reason the resin had to be swelled to insure ready reaction.

To provide a reactive group for attachment of the peptide, the aromatic rings of the resin were partially chloromethylated (*13*) with chloromethyl methyl ether, with anhydrous stannic chloride as catalyst (Fig. 2). Only one ring of the polystyrene is shown, the remainder of the polymer being represented by R. The resulting benzyl chloride derivative could then be converted to an ester by reaction with the triethylammonium salt of the first protected amino acid (Fig. 3). This particular ester bond was selected because it provided a linkage between the peptide and the solid support which was stable to all of the synthetic steps but which could be selectively cleaved after the peptide was completed.

Selection of the protecting group was also of great importance, since the deprotection step had to proceed readily but without splitting of the anchoring ester bond or of other sensitive bonds in the peptide. Most of the original exploratory work was carried out with the carbobenzoxy group (*14*) as the blocking agent for the α-amine function. In standard peptide synthesis the carbobenzoxy group is by far the most widely used, and for several very good reasons. In the present case, however, it was not the best choice because the conditions for its removal by hydrogen bromide were too vigorous; under conditions which effected complete deprotection there was significant splitting of the C-terminal ester bond. The conventional hydrogenation procedures were, of course, not applicable, since the substrate was a solid.

A much better protecting agent was found in the *t*-butyloxycarbonyl group (*15*; Fig. 3). This group was much more readily removed from amino acids or peptides by anhydrous acids than was the carbobenzoxy group. Thus, in 1*N* HCl in acetic acid the *t*-butyloxycarbonyl could be quantitatively removed in a few minutes with essentially no loss of the anchoring benzyl ester bond. The adoption of this protecting group was a very important practical improvement. Another acid-sensitive amino protecting agent which may be appropriate for this application is the *o*-nitrophenylsulfenyl group of Zervas *et al.* (*16*).

Following the cleavage of the *t*-butyloxycarbonyl group by HCl, the *N*-terminal amine was liberated from its hydrochloride with an excess of triethylamine. It was then free to react with the activated carboxyl of the next protected amino acid to form the first peptide bond. A large number of coupling reactions have been developed for peptide synthesis, but only two, the carbodiimide and active ester methods, have been extensively employed in solid-phase peptide synthesis. The requirements were similar to those of standard syntheses except that it was even more important to achieve high yields. Unless a quantitative reaction of the growing peptide chain was obtained at every step, peptides of intermediate length and lacking various amino acid residues would gradually accumulate and contaminate the final product. For this reason a very reactive derivative was required, and it was used in excess to force the reaction of the peptide chain to completion. The excess reagent was then readily removed by filtration after the coupling step was completed.

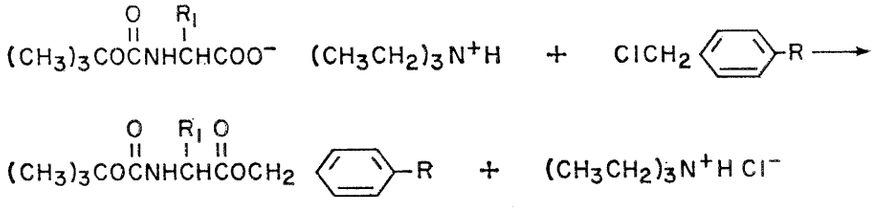


Fig. 3. The esterification step.

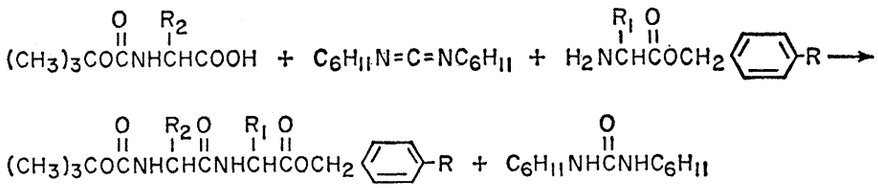


Fig. 4. The peptide-forming step with dicyclohexylcarbodiimide.

Dicyclohexylcarbodiimide, first used by Sheehan and Hess (*17*) for peptide synthesis, proved to be a very satisfactory coupling reagent (*8*). The reaction, shown in Fig. 4, was highly dependent on the proper choice of solvent. Good yields were obtained only in solvents which caused swelling of the resin and which, at the same time, had high dielectric constants. Either methylene chloride or dimethylformamide was effective, whereas benzene, dioxane, ethanol, and water were not. Since dimethylformamide favors a rearrangement to the acyl urea, the preferred solvent was methylene chloride. In this solvent, at room temperature, with a 50-percent excess (1.5 mole) of *t*-butyloxycarbonyl amino acid, the reaction was complete and quantitative in a few minutes. The by-product, dicyclohexylurea, although relatively insoluble in most solvents, was readily removed from the peptide-resin by washing with ethanol and acetic acid. Any contaminating amino acyl urea was also removed at the same time.

Both the *p*-nitrophenyl ester method of Bodanszky (*18*) and the hydroxyphthalimide ester method of Nefkins (*19*) have been satisfactorily applied to solid-phase peptide synthesis. Here, too, the condensation reaction was solvent-dependent. It went rapidly to completion in dimethylformamide, but not in several other solvents. Bodanszky and Sheehan (*20*) have also found the nitrophenyl esters to be applicable to solid-phase peptide synthesis. Although these amino acid derivatives require an additional step in their synthesis, they offer certain compensating advantages. They are especially helpful for introducing asparagine and glutamine into peptides because they do not give rise to the corresponding nitriles (*21*). The latter are produced during carbodiimide-mediated coupling reactions (*22*) and would presumably contaminate the final product if this reagent were employed. Other esters, such as hydroxysuccinimide (*23*) and pentachlorophenyl (*24*), may also be useful activated derivatives for the new method.

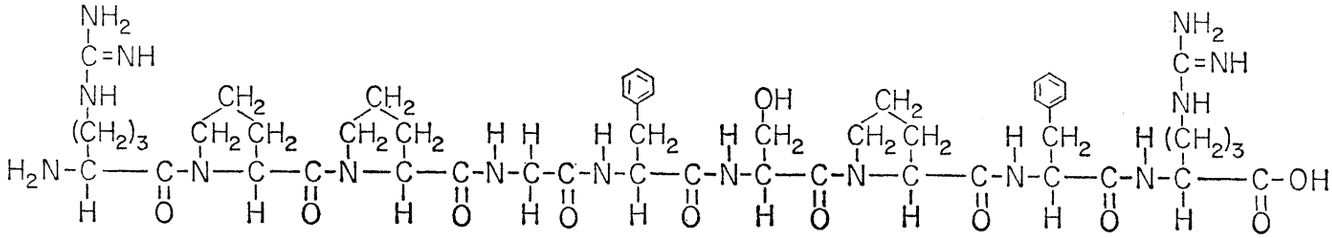
The final step in the synthesis was the cleavage of the completed peptide from the resin. The substituted benzyl ester linkage was chosen because it could be split with anhydrous hydrogen bromide in acetic acid (*25*) or trifluoroacetic acid (*26*). Under these conditions the peptide chain was stable, and racemization was not a problem. Alkaline saponification (*8*), ammonolysis, and hydrazinolysis (*20*) have also been employed for special reasons.

Specific Applications

As the procedures for solid-phase peptide synthesis were being developed they were tested at various stages by the synthesis of several small di-, tri-, and tetrapeptides (*8*). In this way the necessary improvements were gradually introduced. The method as finally outlined above was then tested by applying it to the synthesis of several biologically active peptides.

Bradykinin (Fig. 5) was the first such peptide to be made (*27*). This important nonapeptide was first recognized by Rocha e Silva *et al.* (*28*) in plasma which had been treated with snake venom or trypsin. Its normal physiological function has not been established (*29*), but bradykinin is known to be a powerful hypotensive agent, to increase capillary permeability, to cause bronchial constriction, to cause pain, to cause inflammation, and to cause smooth muscles to contract. The peptide was isolated in pure form by Elliott *et al.* (*30*) and was first synthesized by Boissonnas *et al.* (*31*). The synthesis of bradykinin and especially of suitable analogs is important for the eventual elucidation of the mechanism of action of this plasma hormone. Since bradykinin was a peptide of moderate size and contained the polyfunctional amino acids arginine and serine, as well as three residues of the imino acid proline, it was expected to provide a good test of the new method. Also the specific biological activity offered a sensitive way to assess the identity and purity of the synthetic product.

The synthesis began with *t*-butyloxycarbonyl-nitro-L-arginine which was esterified to chloromethylated copolystyrene-divinylbenzene resin. The protecting group was removed by normal hydrochloric acid in acetic acid, and the resulting hydrochloride was neutralized with triethylamine in dimethylformamide. The free base was then coupled with *t*-butyloxycarbonyl-L-phenylalanine, with dicyclohexylcarbodiimide as the activating agent. These last three steps constituted one cycle of the sythesis, that is, the lengthening of the chain by one amino acid residue. The remaining amino acids were added in a similar manner by repeating the cycle seven more times,



L-ARG-L-PRO-L-PRO-GLY-L-PHE-L-SER-L-PRO-L-PHE-L-ARG

Fig. 5. Bradykinin.

each with the proper amino acid derivative. The final protected bradykinin derivative was *t*-butyloxycarbonylnitro-nitro-L-arginyl-L-prolyl-L-prolyl-glycyl-L-phenylalanyl-*O*-benzyl-L-seryl-L-prolyl-L-phenylalanyl-nitro-L-arginyl-copolystyrene-divinylbenzene. The peptide was cleaved from the solid support with anhydrous hydrogen bromide in trifluoroacetic acid. This reagent also cleaved the *N*-terminal *t*-butyloxycarbonyl group and the ether bond in the *O*-benzyl serine residue, but did not remove the nitro groups from the two arginine residues. The nitro groups were removed by hydrogenolysis with palladium black catalyst, giving the free peptide. The question then arose as to whether or not the product was actually bradykinin and whether or not it represented a good yield of pure, biologically active peptide. Had each of the eight deprotection, neutralization, and coupling steps been quantitative, had racemization been avoided, had the ester bond survived these procedures, had the washing procedure provided effective purification at all the intermediate steps, and had the peptide been cleaved from the resin in good yield without damage to the rest of the molecule? These questions were answered affirmatively in the following ways. The crude free peptide was passed through an ion-exchange column, and 93 percent was found in a single component. This fraction was isolated in 68-percent overall yield and shown by paper chromatography, paper electrophoresis, and countercurrent distribution to be homogeneous and indistinguishable from authentic bradykinin. It was also judged to be pure by amino acid and elemental analyses and by optical rotation. The preparation possessed the full activity of bradykinin when compared quantitatively with a standard in the rat-uterus assay and in the rat-duodenum assay.

This demonstration that the solid-phase method could be satisfactorily applied to the synthesis of bradykinin has led to its use in the synthesis of a considerable number of related compounds. It was, for example, applied to the synthesis of methionyl-lysyl-bradykinin (*32*), a new plasma kinin isolated by Elliott *et al.* (*33*). The biologically active peptide was prepared by the same general procedures just described. It was necessary to protect the ϵ-amino group of lysine with a carbobenzoxy group and to protect the sulfur of methionine from alkylation by effecting the HBr-trifluoroacetic acid cleavage in the presence of methyl ethyl sulfide. The isolated undecapeptide was shown to be formed in good yield, to be chemically pure, and to possess biological activity comparable to that reported for the natural kinin.

In their efforts to prepare inhibitors of bradykinin Stewart and Woolley (*34*) have utilized the new method to great advantage. It has enabled them to synthesize a large number of pure analogs in a relatively short time with a minimum of effort. The interesting "all D" bradykinin (*35*) was made in this way. Its physical and chemical properties were entirely comparable to those of its natural enantiomorph. The synthesis of 22 other new bradykinin analogs (*34*) led to the discovery that replacement of the two residues of phenylalanine by *O*-methyl tyrosine would give a compound which was inhibitory at low concentrations.

As a further test of the applicability of solid-phase peptide synthesis, the preparation of angiotensin II (*36*) (Fig. 6) was undertaken recently (*37*). The synthesis of this important hypertensive octapeptide was of interest because it was anticipated that, once this had been achieved, the new method would provide a rapid and simplified route to various analogs of the hormone, just as it had in the case of bradykinin. In addition, angiotensin provided a further test of the method because it contained five amino acids which had not previously been introduced into peptides in this way. For the new method to be of real value it must be able to cope with all of the amino acids.

The general approach was exactly as before. All the amino acids were used as $N(\alpha)$-*t*-butyloxycarbonyl derivatives, with the following additional protecting groups for the trifunctional amino acids: tyrosine-*O*-benzyl ether, imidazol-benzyl histidine, nitroarginine, and β-benzyl aspartate. They were all coupled by the dicyclohexylcarbodiimide procedure. The protected octapeptide was cleaved from the resin support by HBr, with simultaneous loss of the benzyl ether and benzyl ester. The benzyl histidine and nitroarginine residues were finally deprotected by hydrogenolysis over a palladium-$BaSO_4$ catalyst. A 56-percent overall yield of pure homogeneous 5-isoleucine-angiotensin II was isolated. The product had full biological activity in the rat-uterus assay.

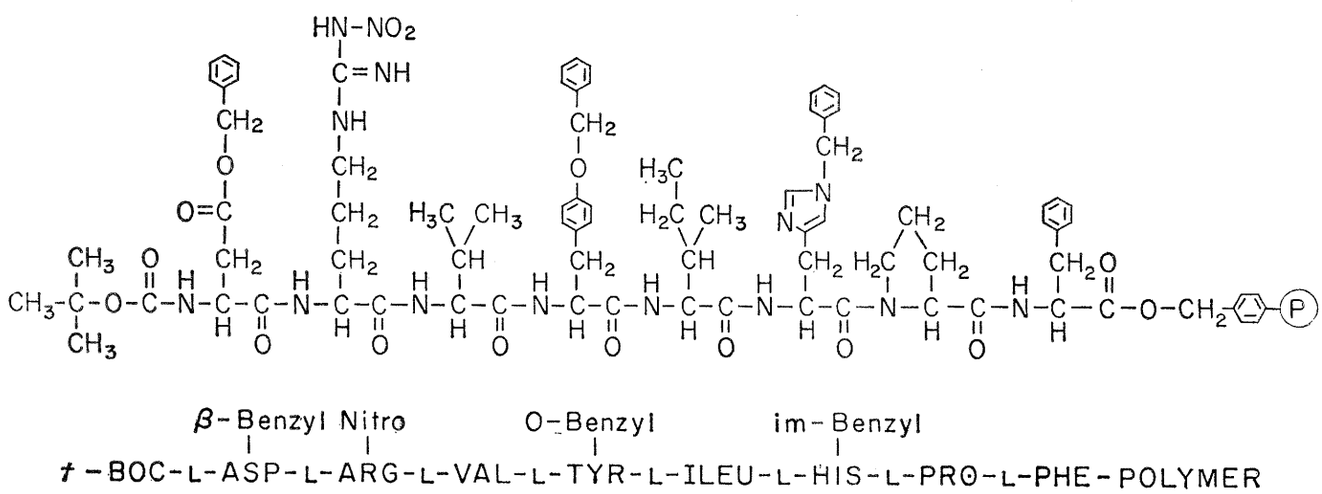


Fig. 6. Protected 5-isoleucine-angiotensin II.

In a similar way 1-asparagine-5-isoleucine-angiotensin II (*38*) was synthesized (*37*). In order to prevent nitrile formation the asparagine was introduced into the peptide chain by the nitrophenyl ester procedure.

It was of great importance to prove that the conditions of the method did not promote the well-known $\alpha \rightarrow \beta$ rearrangement of aspartic acid–containing peptides. Page (*39*) had shown that mineral acid converted angiotensin to β-aspartic angiotensin, in which the normal peptide bond had rearranged to give an amide linkage containing the β-carboxyl group. Riniker and Schwyzer (*40*) had shown that boiling water would also effect the isomerization, and it was known from earlier work (*41*) that alkaline conditions rapidly established an equilibrium between the two forms by way of an intermediate imide. With the aid of model tri- and tetrapeptides containing aspartic acid benzyl ester it was found that neither the HCl-acetic acid nor the HBr-trifluoroacetic acid steps caused the rearrangement, but that NaOH, as expected, did produce equilibrium proportions of approximately 80 percent β and 20 percent α. Finally, it was clearly demonstrated by several tests that the angiotensin synthesized by the solid-phase method was, itself, completely free of the β derivative.

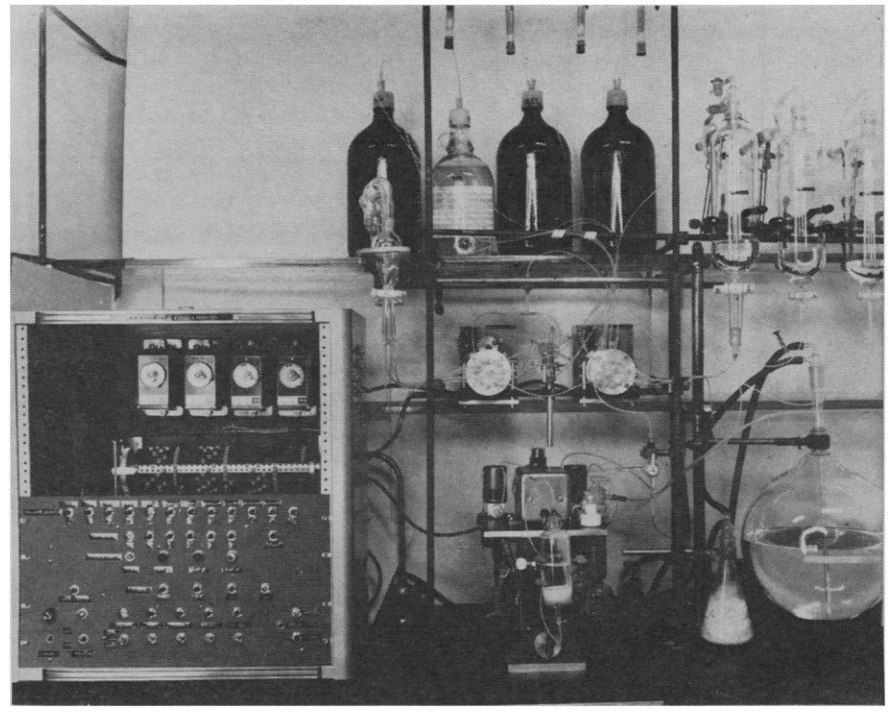


Fig. 7. The instrument designed for automation of peptide synthesis.

Racemization

Throughout the history of peptide synthesis one of the problems of major concern has been that of racemization. The retention of configuration, especially in the synthesis of biologically active peptides, is essential. On the basis of the available data (*42*), the generally held view is that there can be some degree of racemization by every known coupling method except the azide. On the other hand, when the proper conditions are chosen and when properly acylated amino acids (rather than peptides) are activated, many of the coupling methods give zero or negligible amounts of racemization.

In an effort to assess this problem from the point of view of solid-phase peptide synthesis a number of tests have been made. In an experiment with L-alanine it was shown that neither the preparation of the *t*-butyloxycarbonyl amino acid, the esterification of it to the resin, nor its cleavage from the resin caused measurable racemization. The specific rotation of the isolated alanine was exactly the same as that of the starting material. In certain instances the specific rotation of the final peptide could be compared with that of the product from a standard procedure which was known to give high optical purity. For example, the tetrapeptide L-leucyl-L-alanyl-glycyl-L-valine had the same specific rotation (*8*) when made by the new method as it did when made by standard procedures which employed the nitrophenyl ester method. Likewise the rotation of synthetic 5-isoleucine-angiotensin II was close to the reported value (*43*), and the synthetic D-bradykinin had a specific rotation equal to, but of opposite sign from, that of L-bradykinin made by conventional procedures (*44*). A third kind of evidence for lack of racemization was the quantitative agreement between the biological activity of synthetic and natural bradykinin and between that of synthetic and natural angiotensin. Although the close correspondence in activity is very satisfying it cannot be considered as proof of identity, since examples are known in which peptides containing D amino acid residues were still biologically active. In some instances the potency was less than that of the natural hormone (*45*), but in others it was considerably greater (*40*, *46*). The most satisfactory test of optical purity is degradation by proteolytic enzymes which are specific for the L isomers. For example the tetrapeptide L-leucyl-L-alanyl-glycyl-L-valine, which had been made by this method, was quantitatively degraded by leucine aminopeptidase. So also were L-leucyl-L-aspartyl-L-alanyl-L-valine and L-aspartyl-L-alanyl-L-valine, but not the corresponding β-aspartyl peptides. Bradykinin is known to be completely resistant to the enzyme, but L-methionyl-L-lysyl-bradykinin gave one mole each of methionine and lysine. The first five residues of synthetic angiotensin were also liberated quantitatively in this way, the histidyl-proline bond being resistant, as expected.

The accumulated evidence thus was very strong that significant racemization had not occurred in the solid-phase syntheses examined so far. One reason was the coupling of activated amino acids by the stepwise procedure, rather than the coupling of activated peptides. Another was the use of the *t*-butyloxycarbonyl group, which appeared to be just as effective as the carbobenzoxy group in preventing racemization. A third contributing factor was the complete absence during the coupling step of chloride ion (*47*) or of tertiary amine (*48*), both of which have been shown to promote racemization in certain cases. Whether

or not the steric restraints imposed by the solid support exerted an influence in this respect is not known.

Automated Peptide Synthesis

The ultimate objective of this work has been, from the outset, to develop a simplified and accelerated system of peptide synthesis which could be automated. The original decision to synthesize the peptide chain on an insoluble solid support was based on the idea that such a device would provide the best chance of accomplishing that objective (*4*). The advantages of the approach and the features which specially adapt it to automation have already been discussed. One of the most important factors was that the synthetic scheme allowed all the chemical reactions to be performed in a single reaction vessel and, in addition, all the intermediate purification steps could be carried out in the same vessel. Therefore, once the supporting resin containing the C-terminal amino acid was in place, it was never removed from the reaction chamber during the entire synthesis. Thus, the manipulations required for the synthesis of a polypeptide chain consited simply of pumping the proper solvents or reagents into and out of the vessel in the proper sequence at the proper times. An apparatus was constructed which performed all these operations automatically (*49*). It is illustrated in Fig. 7. The solvents and reagents, stored in reservoirs at the upper right, were transferred through an all-glass-and-Teflon system by means of a metering pump into the glass reaction vessel. After mixing for a suitable time the solvent was removed by filtration through the fritted disc at the bottom, and the next solvent was pumped in. The key components in this operation were two motor-driven circular selector valves, designed and constructed by Nils Jernberg. During one complete cycle of the synthesis the first valve made one complete revolution of 12 steps and thus selected in sequence each of the reagents and solvents required for the lengthening of the peptide chain by one amino acid residue. The correct amino acid solution for each succeeding cycle was selected by a second rotary valve which advanced only one position for each cycle of the synthesis.

These events were controlled by the programmer shown at the left in Fig. 7. The 80 separate operations of one cycle were selected in proper sequence by appropriately positioned pins on the control drum. The drum was signalled to advance from one position to another by suitable timers. At the end of one cycle the drum automatically reset and began the next cycle, which consisted of exactly the same series of events, except that a new amino acid was introduced. In this way six amino acids could be added to the peptide chain during a 24-hour period without manual attention. To continue the synthesis, the reservoirs were rinsed, refilled with the next six amino acids, and arranged in their proper sequence in the rack. Two interchangeable programmed drums were used, one for the carbodiimide-mediated coupling steps and one for the active ester coupling reactions. Either of these programs could be readily modified to conform to changes in the chemistry or reaction conditions of the process by repositioning the pins on the drums. The instrument was first tested by repeating the solid-phase synthesis of bradykinin by the same series of reactions as described above for the manual procedure. The synthesis of the protected nonapeptide chain was completed in 32 hours of continuous operation of the machine. The final purified peptide was obtained in comparable yield and purity and had full biological activity. The saving in the overall time and effort expended in the synthesis of this relatively small peptide was significant, and it is expected to become a progressively more important advantage as the length of the peptide chain is increased. With this accelerated system available a practical way is now open to the synthesis and study of large polypeptides and perhaps even of proteins.

Summary

Peptides have been synthesized by a new method which depends on carrying out the synthetic reactions while the growing peptide chain is bound to an insoluble supporting solid; the method is therefore called "solid-phase peptide synthesis." The idea was originally conceived and developed because it offered distinct advantages in yield, ease of operation, and speed, and these particular qualities were necessary for the eventual automation of the process. An instrument has recently been constructed which performs and controls automatically all the operations necessary for the synthesis of a peptide. The method has been successfully applied to the synthesis of several biologically active peptides, including bradykinin and angiotensin. Extension of this approach to the synthesis of much larger peptides and possibly even to proteins is predicted.

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The Rubidium Magnetometer in Archeological Exploration

Exploration with a highly sensitive magnetometer allows use of more effective survey techniques.

Sheldon Breiner

By shining a purple light through an empty bottle, we may greatly increase our chances of finding ancient buried walls and pottery. The purple light and empty bottle in this case, however, are the working parts of a rubidium magnetometer recently demonstrated to be effective for delineating buried structures and potsherds near the possible site of the ancient Greek city of Sybaris. The application of this instrument to archeological exploration opens many new facets of magnetic search techniques, for it is more than 100 times as sensitive as the magnetometers used earlier.

The importance of developing methods for locating buried sites and objects of archeological interest is recognized even by the nonarcheologist. The cut-and-try method of excavating at random will simply not work in many areas where surface evidence is completely lacking. Several methods are available to the archeologist, however, to aid him in the search: (i) search of historic writings; (ii) systematic drilling, dredging, or trenching; (iii) analysis of aerial photographs; and (iv) geophysical methods based on seismic, electrical, or magnetic survey techniques (*1*). It is with the last method that we are concerned.

After the development in 1955 of the first directionally independent and truly mobile instrument for making magnetic surveys, the proton magnetometer (*2*), the speed and ease with which we were able to perform such surveys were vastly increased. Since that time reports have appeared describing the use of the proton magnetometer for detecting buried kilns, tombs, walls, and forts (*3, 4*). A portable instrument such as the Elsec proton magnetometer (*1*) can resolve a change of approximately 1 gamma (10^{-5} oersted) where the earth's total magnetic field intensity is 50,000 gammas. This high accuracy and mobility are achieved through use of the phenomenon of nuclear magnetic resonance known as free precession. The spinning protons in a fluid such as water behave like small, randomly oriented bar magnets. Through application of a uniform magnetic field about the sample, the protons are aligned in the direction of the artificial field. When the applied field is removed, the protons precess about the direction of the ambient magnetic field at a frequency proportional to the field intensity. The measurement of this frequency, however, has two practical limitations. First, the maximum sensitivity of a portable proton instrument is about 1 gamma. Also, in order to polarize the protons, the instrument must operate discontinuously. The portable versions require from 4 to 6 seconds per reading.

In 1957 H. G. Dehmelt (*5*) described a method for optically pumping and monitoring the energy-level states of electrons of the alkali metals. Optical pumping is a method by which electrons are caused to undergo selective energy-level transitions and to become concentrated in a particular energy sublevel of the atom (*6*). This method provided a means for monitoring the transition frequency of atoms with much greater sensitivity to magnetic field intensity than could be achieved with the proton precession method. Moreover, the process was continuous and well suited for mobile sensing of the field. These principles were applied in the development of a magnetometer in which was used, among other elements, the vapor of rubidium. The U.S. Navy and the National Aeronautics and Space Administration funded the manufacture of such instruments for geophysical applications; NASA used the principles for rocket and satellite measurements of field intensity. Instruments

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