*APPLICATION OF A THEORY OF ENZYME SPECIFICITY TO PROTEIN SYNTHESIS**

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The necessity for "activation" of an amino acid to allow it to form a peptide bond has two aspects. The first of these is thermodynamic and arises from the fact that the equilibrium in the reaction shown in equation (1) lies far to the left:

$$^{+}H_3N{-}CHRCOO^{-} + \overset{+}{H_3}NCHR'COO^{-} \rightarrow \overset{+}{H_3}N{-}CHRCO{-}NHCO\overset{-}{O} + H_2O \quad (1)$$

The suggestion of Lipmann[1] that the energy required to drive this reaction to the right came from adenosine triphosphate has been supported by the extensive work[2] which has been discussed by the previous speakers on this symposium. While the detailed mechanism of this energy transfer has not been fully elucidated, the ATP apparently forms an amino acid anhydride. The fact that an anhydride of relatively high-energy content is the method of driving the reaction thermodynamically fits in with organic practice in which acyl anhydrides are the usual reagents for acylations.

In addition to thermodynamic activation, there is a needed "kinetic activation." For example, the acyl phosphates and acyl adenylates acylate amines non-enzymatically at appreciable rates in aqueous solution,[3] but this reaction is certainly not rapid enough or selective enough to account for protein synthesis. The reaction of these acyl anhydrides to form peptides must be catalyzed, and it is this catalysis which is the subject of this paper.

An analysis of why this linking of amino acids presents such formidable difficulties is revealing. The difficulty appears to be caused by a combination of requirements, each of which, taken singly, is rather easily satisfied. The first requirement is that an individual position in the protein be occupied by one and only one amino acid. This by itself is not a difficult condition to satisfy, since enzymatic reactions of equally high specificity are well known. The second requirement is that a high-molecular-weight polymer be produced. Again, this, by itself, is not a unique condition, since the enzymatic formation of high-molecular-weight molecules from a given monomer is also familiar, e.g., carbohydrate polymerization catalyzed by phosphorylases. Finally, the macromolecule is formed from many different monomer units, but this requirement is also easily achieved if the units are randomly arranged, e.g., polynucleotide formation by polynucleotide phosphorylase.[4] However, the combined requirements, i.e., the synthesis of a macromolecule from many individual monomers to give a single specified sequence, is a problem of different magnitude. A mechanism involving an individual enzyme for each bond would be acceptable from the specificity point of view, but it would require an inconceivably large number of enzymes to form all the proteins of the cell. Moreover, there are other observed conditions, e.g., the necessity of feeding all the amino acids

within a short period of time[5] and the lack of intermediates,[6] which argue against a sequence of free enzymatic reactions like those in carbohydrate metabolism.

The best framework for explaining at least part of these phenomena has come from a mechanical analogy, i.e., the template. This analogy has been refined over the years, and recently Dalgleish has proposed an ingenious modification[7] which is able to account for both asymmetric labeling of a protein[8] and a rapid rate of synthesis. He suggests that the part of the peptide chain which has been completed peels off the template as it is formed, allowing new peptide chains to be started before the initial one is completed. Thus the time for formation of an individual protein molecule is extended, while the high number of molecules formed per unit time is maintained.

The deficiency of any template model is simply that it is only a mechanical analogy and, since we are making chemicals and not automobiles, that it has to be translated into chemical terms. For example, in the model of Dalgleish it is assumed that the completed peptide peels off the template; but why individual amino acids which were previously tightly adsorbed should suddenly prefer to be desorbed must await chemical reasoning. The first step in this reasoning is an attempt to deduce the nature of the template itself.

The evidence seems overwhelming that the template has the characteristics of an enzyme, since the three features cited above are observed singly in enzyme-catalyzed reactions. Certainly, inductive effects or van der Waals attractions would not account for the exclusive selection of isoleucine in competition with valine or serine in competition with threonine at a specific position in a peptide chain. Whether these interactions are provided by a protein or by a nucleic acid or by a combination of the two cannot be decided as yet, but the monodisperse nature of the peptide chains suggests that the thermodynamically activated amino acid anhydride is kinetically activated by a catalyst which behaves like, even if it is not identical with, an enzyme.

Let us, therefore, first examine the manner in which an enzyme exerts its specificity. It is apparent that this specificity does not arise from inductive effects in the substrate molecules. A change from a hydroxy to a methoxy group at the C-3 position of glucose will have negligible electrostatic effects on the basic or acidic properties of the C-6 hydroxyl, and yet this change is enough to block completely the action of hexokinase.[9] To explain this specificity, a "key-lock theory" was proposed.[10, 11] In essence this theory said that the enzyme was a rather rigid negative of the substrate and that the substrate had to fit into this negative to react. Hence a modification which had minor electrostatic effects but which would so increase the bulk or change the shape of the molecule that it could not fit on the enzyme surface would prevent reaction. This explanation is supported by the demonstration of steric repulsions in organic reactions and by the fact that the kinetics of enzyme action are compatible with an enzyme-substrate complex. It is capable of explaining almost all the observed specificity patterns.

On close examination,[12] however, it is clear that this hypothesis does not explain all cases, and, as usual, it is the anomalies which lead to revisions of our theories. The anomalies fall, in general, in the class that smaller analogous compounds react extremely slowly or not at all. For example, ribose-5-phosphate is hydrolyzed by 5′-nucleotidase much less rapidly than adenylic acid.[13] Since the ribose-5-phosphate

is simply adenylic acid without the purine, it could be argued that an attractive group is eliminated and hence the affinity to the enzyme is less (which it probably is). However, when this lower affinity is compensated for by comparing the velocities at enzyme saturation, the ribose-5-phosphate still reacts at $^1/_{100}$ the rate of adenylic acid. The key-lock theory would certainly predict equal rates at saturation levels. Similarly, the failure of alpha methyl glucoside to act as a substrate for amylomaltase[14] is inconsistent with this hypothesis. Alpha methyl glucoside has the same structure as the natural substrate maltose, except that it is smaller, i.e., two hydrogens on the aglycon carbon take the place of the second pyranose ring. As would be expected from its smaller, but otherwise similar, structure, alpha methyl glucoside should be absorbed on the enzyme surface. That it does is shown by its action as a competitive inhibitor. However, it is not hydrolyzed by the enzyme. In this case certainly, lack of access to the enzyme surface cannot explain its failure to be a substrate. Numerous similar anomalies can be found.[12]

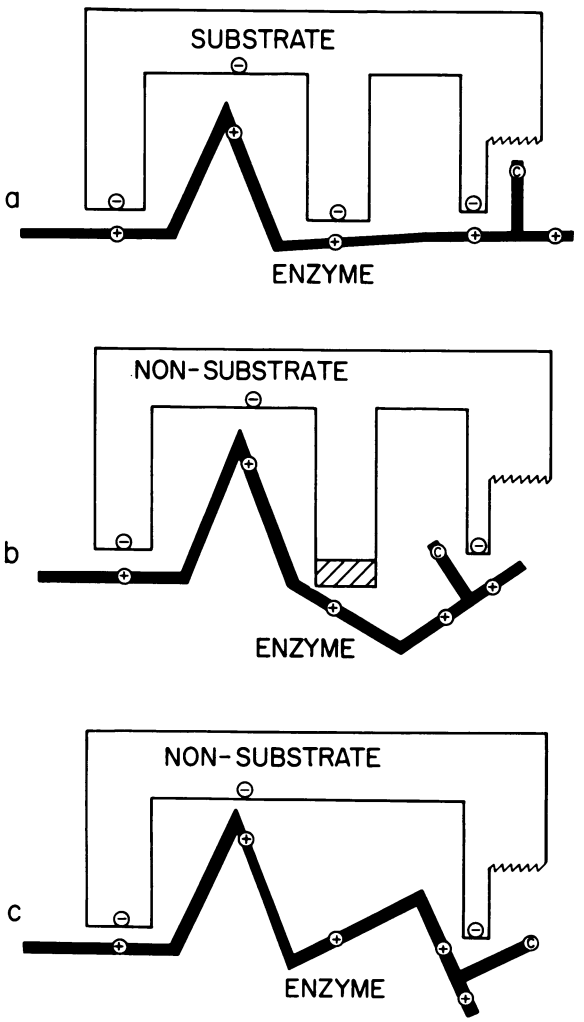

FIG. 1.—Interaction of enzyme with (*a*) substrate, (*b*) compound too large to be substrate, and (*c*) compound too small to be substrate. Circled pluses and minuses indicate any mutually attractive groups on enzyme and substrate. Circled *C* stands for catalytic group, and jagged line for bond to be broken.

The explanation that we suggest to explain these phenomena is as follows: (*a*) a precise orientation of catalytic groups is required for enzyme action; (*b*) the substrate may cause an appreciable change in the three-dimensional relationship of the amino acids at the active site; and (*c*) the changes in protein structure caused by a substrate will bring the catalytic groups into the proper orientation for reaction, whereas a non-substrate will not. This set of postulates has been called "the induced fit" theory for brevity and to emphasize that, while the idea of a fit is retained from the key-lock theory, the fit in this case occurs *only after* the changes induced by the substrate itself.

A simplified schematic representation is shown in Fig-

ure 1. In Figure 1, *a*, a catalytic group, *C*, is aligned with the bond to be broken (shown by the jagged line). In Figure 1, *b*, increasing the size of a group well removed from the bond to be broken destroys the alignment; and in Figure 1, *c*, the complete removal of the same group likewise destroys the alignment. Thus this theory explains why a substrate can be converted to a non-substrate by a decrease in, as well as by an increase in, its size.

Furthermore, there is independent support for the various postulates. First, the flexible nature of portions, if not all, of the protein chain is adduced from many sources, and these changes can be caused by small molecules, charged or uncharged. A typical example is the reversible denaturation of a number of enzymes by urea.[15] This denaturation is accompanied by changes in viscosity, optical rotation, and sedimentation constant, which certainly are indicative of an alteration in the geometry of the amino acids of the protein relative to each other. The urea undoubtedly causes these changes by competing for the internal hydrogen bonds of the protein.[16] The reversible nature of this denaturation shows that, upon removal of urea, the protein returns to its natural conformation. The urea, because of its particular structure, is attracted non-specifically to many peptide links, whereas a hydrogen-bonded substrate is attracted to only one or a small number of sites. It is certainly reasonable, however, to expect that the specifically attracted substrate will cause similar conformation changes in its immediate environment. Charged or hydrocarbon-type compounds are observed to cause analogous alterations in protein structure.

Second, there is support for the postulate that a precise orientation of catalytic groups is important in enzyme catalysis. The evidence comes from analogies to both heterogeneous and homogeneous catalysis in simple chemical systems and also from the observed behavior of the enzymes themselves.[17] Finally, the idea that the substrate is necessary to promote the proper orientation of catalytic groups can explain phenomena other than their specificity, for example, substrate-promoted isotopic exchanges.[18]

Provocative suggestions for translating the template into chemical terms can be derived from the induced-fit theory of specificity. For example, Figure 2 shows a possible sequence of reactions in the formation of a peptide bond in the center of a protein molecule. In this illustration, each activated amino acid can form an acyl bond with its appropriate position on the synthesizing template. However, we again assume that this template is not initially in its proper orientation but that this orientation is induced by the presence of the previous amino acid. Thus in Figure 2, *a*, the tenth amino acid has formed an acyl bond and hence has brought position 11 into the proper alignment (schematically indicated by being on a horizontal straight line) to attack the acyl group of the amino acid anhydride,

$$\begin{array}{l} \quad\quad O \\ \quad\quad \| \\ X\text{—}C\text{—}CHR_{11}\text{—}NH_3^+. \end{array}$$

After formation of the acyl-template bond (Fig. 2, *b*), the amino group is in the appropriate position to attack the carbonyl carbon of the tenth amino acid. As this peptide bond is formed (Fig. 2, *c*), the completed portion of the peptide peels off, and position 12, which was previously unreactive, is brought into proper orienta-

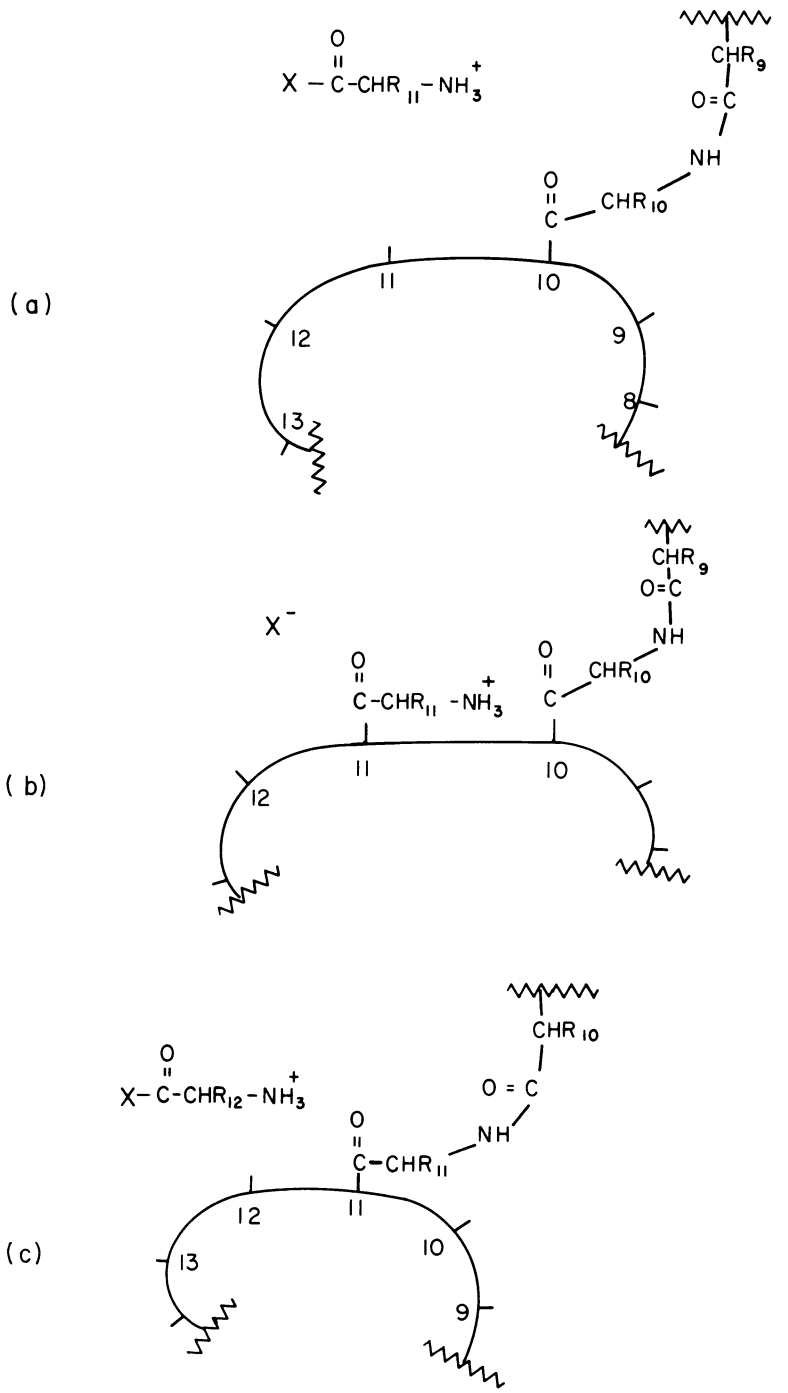

FIG. 2.—Possible sequence of events in protein synthesis. (*a*) eleventh activated amino acid approaches template, in which eleventh site has been "induced" into reactive position; (*b*) acyl-template bond is formed, and favorable geometry for reaction between eleventh and tenth amino acids established; (*c*) formation of peptide bond between eleventh and tenth amino acids induces site 12 into reactive position and allows completed portion of peptide to peel off template.

tion for reaction with its activated amino acid. Persumably in this case the last position would be specific for a simple amino acid, i.e., having a free carboxyl group, which then detaches the completed peptide chain from the template when its amino group is acylated.

Although a covalent bond was shown in the illustrative example, other permutations are possible. The attached, e.g., tenth, residue of the peptide might be present as a tightly bound adenylate. As the next, i.e., eleventh, acyl adenylate arrives, its amino group reacts to form a peptide bond, and its adenylated carboxyl group is then tightly bound. Or the tightly bound groups could be at the amino end of the peptide, in which cases elongation of the peptide would occur by displacement by this amino group in the carbonyl of the incoming amino acid anhydride. The key features of the mechanism are, therefore, that the end of the completed portion of the peptide chain is tightly bound to the template; that this tight bond is released upon formation of the peptide bond with the subsequent amino acid; and that each peptide bond formed "induces" a new template area, which allows the formation of the next bond.

This chemical model is consistent with the template model. It explains how a zipper-type action could occur, since each link added to the chain establishes the conditions for the next link. It explains how the peptide chain can peel off the template as each link is completed. The peeling-off occurs because the formation of the peptide bond causes a dramatic change in chemical type. In the example shown, the covalent bond is broken. In the case of a strongly attracted amino acyl-X or $-\overset{+}{N}H_3$, either the X is released or the positive charge is removed. Hence a template affinity strongly dependent on X or the positive charge would be eliminated. As a result, the intramolecular attractions in both the template and the completed peptide would probably become more important than the template-peptide attraction. The peeling-off of the peptide chain and the return of the template to its original condition then allow the initiation of new chains before the completion of the old.

This model also explains other phenomena which are not a necessary concomitant of the zipper concept. For example, a zipper-type model does not provide any necessary reason for the absence of intermediates on failure to supply one amino acid. There would be no reason to keep the template from linking residues until a position requiring the deficient one was reached, in which case the partially completed peptide chain would accumulate. However, the concept that the growing end of the chain is always tightly bound to the template unless released by the subsequent amino acid means that a deficiency would leave the template "poisoned" by its own partially completed chain. Thus intermediates could only accumulate in amounts equal to the concentration of template, which is very small. Moreover, the chain could not start in the middle and proceed to the end, since the initiating site has not been "induced" into its proper orientation.

Finally, it should be stated that the author is well aware that this theory is not a complete answer to protein synthesis. It is, however, as far as he knows, compatible with the existing facts and leads to concrete predictions, e.g., under conditions where asymmetric labeling is observed, a particular amino acid should have progressively increasing specific activity on going down a chain. Also the combination of

data from the action of pure enzymes with data on timing in in vivo systems puts some chemical flesh on the mechanical skeleton of the template.

Summary.—The problem of protein synthesis is formidable because of a combination of three requirements, each one of which by itself is rather easily satisfied. Since the conditions are satisfied in each case by an enzyme-catalyzed reaction, the "template" in protein synthesis is presumed to be enzyme-like in character. Recently a new theory to explain enzyme specificity has been proposed which suggests that reaction between enzyme and substrate can occur only after a change in protein structure induced by the substrate itself. When this theory is applied to the problem of protein synthesis, it is seen that the existing data can be explained by a flexible template in which each completed peptide bond induces an alignment necessary to the formation of the next bond.

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