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# Some Observations on Protein Biosynthesis

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Dedicated to Professor *Ole Lamm* on his 60th birthday

It has been pointed out that the high entropy change in protein biosynthesis necessitates another synthesis mechanism than that for the synthesis of the usual di- or tripeptides, where the entropy effect is not so large. The possibility of an upper limit for one step formation of a peptide chain is discussed. In consequence of this, longer peptide chains, as in collagen, must be built in two steps.

Proteins are polymers of amino acids, but they are polymers which are closely defined as to structure and molecular weight. Studies of the amino acid sequences of proteins indicate a remarkable specificity in the sequences, indicating a well organized biosynthesis machinery. During recent years the biosynthesis of proteins has been a main interest in biochemistry since ways have been found to elucidate some features of the process.

The biosynthesis can be divided into three different main steps. The first stage consists in the sending out of information from the nuclear part of the cell to the synthesis mechanism as to how the protein should be built up. The second part is the synthesis of the peptide chain or chains which build the protein, and the last step is the formation of the protein from the chains. The discussion here concerns mainly the second stage, *i.e.* the biosynthesis of the peptide chain.

Studies of Hoagland [1] and others show that the first step in the route of amino acid incorporation is that the acids are esterified to ATP * by special enzymes. There are as many different enzymes performing this esterification as there are amino acids. When studying the incorporation of labelled amino acids Hoagland *et al.* [2,3] found that the amino acids were then transferred to a ribonucleic acid from the soluble fraction (s-RNA). Even here there is one kind of specific acceptor for each amino acid (Leahy *et al.*[4]). The amino acid is bound terminally to s-RNA by an ester linkage (Zachau *et al.*[5]). From the work

* Abbreviations: ATP, adenosine triphosphate; GTP, guanosine triphosphate; s-RNA, soluble ribonucleic acid.

of Zamecnik *et al.*[6] it is known that the microsome particles (called ribosomes) are involved in the last step where the peptide synthesis occurs. The ribosomes contain on the surface nucleic acid, which, together with the s-RNA, might give the right sequence. Not much is known about the condensation mechanism which is independent of the sequence giving factor. It has been shown that GTP takes part in the condensation (Keller and Zamecnik[7]) but the extent to which it has broken down is unknown as specific systems have not been used. A transferring enzyme, having an SH-group, is known to exist. The author has shown that vitamin C cooperates with it [8].

It seems that a distinction should be made between protein synthesis and polypeptide synthesis when investigating *in vitro*. Protein synthesis will be defined as a synthesis performed in such a way that every amino acid occurs in its right place in the sequence as determined by the information given for the protein. Polypeptide synthesis will be an incorporation of amino acids more randomly. This distinction means that it is not always enough to show that a tracer amino acid has been incorporated with peptide bonds in a chain. It ought also to be shown that it is in its right site in the sequence.

Lipmann [9] has shown that labelled leucine esterified to s-RNA of bacterial origin, together with ribosomes from liver, shows an incorporation of the labelled leucine in peptide bonds on the ribosome. He remarks on the low specificity of the bacterial s-RNA in synthesis reactions. However it seems that in this case one is dealing with a polypeptide synthesis and that this can explain the low specificity of the bacterial s-RNA. The peptide bond synthesis is independent of the ordering factor and it is possible to perform the peptide bond synthesis with any amino acyl residue. It may thus happen now and then that an amino acyl ester will come in such a position that a peptide bond synthesis can occur without any ordering factor taking part in the process. The rate of synthesis *in vitro* is very low, being about 0.001 of that *in vivo*, and in many cases where the ratio between s-RNA amino acids and ribosomes is unfavorable only polypeptide synthesis is obtained. It is certain that *in vivo* factors will be found regulating the ratio between s-RNA and ribosomes so that the order will not be disturbed.

As can be seen, protein synthesis requires a large amount of energy. The energy for inserting each amino acid in the peptide bond chain is taken from ATP and GTP. The peptide bond in a chain has, however, a low value of free energy (Borsook [10]). This very great discrepancy depends of course on the very high change in entropy occurring when a system with a high degree of order is obtained. The consequence of this is that the enzymatic processes as they usually occur cannot be used here. Often only one choice of combination is used and the entropy change is not of this magnitude.

It is therefore not possible to study the synthesis of peptide bonds using only one choice, as in hippuric acid and glutathione, and expect to obtain any information of great value about protein synthesis. In such cases the synthesis mechanism is quite different and it would be a waste of energy to use there the process of protein synthesis. In the case of the former synthesis the principle of active surfaces can be used to lower the energy of activation of the reaction. In the ordinary reactions the enzyme thus tunnels under the energy pass between the reactants and the results of the reaction.

The energy of activation is, according to Eyring [11], a function of the entropy, and if the change is large the energy of activation cannot be lowered appreciably. It is then quite understandable that the energy providing reaction in protein synthesis must be quite unspecific as concerning amino acids. The different amino acids have nothing in common except the two groups taking part in the reaction. The usual principle of active surfaces for the enzyme cannot be used between the amino acids and the enzyme cannot directly take part in the coupling process. It can only release and transport energy for the process. It takes part in an activation process of the $\alpha$-amino group. In this process the enzyme cooperates with dehydroascorbic acid forming a complex which is the active enzyme. The $\alpha$-amino group is phosphorylated to a high energy compound by transfer of pyrophosphate from guanosine triphosphate [12]. Both amino and imino groups can be phosphorylated while both amino and imino acids are incorporated in peptide chain. It is shown by the synthesis of poly-$\alpha$-amino acids from their methyl esters that the reaction is unspecific as far as the type of esterbonds is concerned [13].

The phosphorylation can only be done if the amino group is unionized. Thus the start of the synthesis is a problem, because at the pH where the reaction occurs the amino acids and most dipeptides have their amino groups ionized and only longer peptides have sufficiently low p$K$ values of their $\alpha$-amino groups. The hormone thyroxine, however, is an exception in a low enough p$K$ value of its $\alpha$-amino group to react [14]. In a reaction mixture with tyrosine methylester as a substrate no polyamino acid is synthesized unless thyroxine is added as an initiator of the synthesis [13].

Thus, in the synthesis of the peptide chain both vitamin C and the hormone thyroxine take part.

Concerning the biosynthesis of proteins the following question will at last be discussed: How large is the largest peptide that can be synthesized in one step of the synthesizing machinery?

The biosynthesis occurs on the surface of the ribosomes. If it is now supposed that every ribosome is an independent synthesizing site some very rough estimate can be made. The ribosomes have, during recent years, been studied quite a lot with different physicochemical methods. They seem to be built up of smaller units which are cemented together by magnesium ions. The largest particles have a diameter of about 150 Å and it is such particles which will be found on the microsomes. The synthesis takes place on the surface of these larger ribosome particles. If the whole surface were covered with amino acids, each taking up a space of 34 Å$^2$, a peptide chain of a molecular weight of 450 000 could be synthesized. Now only a small part of the surface can be free for the peptide chain, because ribonucleic acid takes up most of it and even other substances adhere. A very rough estimate shows that less than one tenth can be occupied by the chain. Thus the upper limit of the molecular weight is 45 000.

Without laying any greater weight on the value it can be supposed that a comparatively low value can be estimated as the upper limit for the synthesis of a peptide chain in one step. This can give some conclusion about the structure of proteins.

It is to be expected that many of the proteins with higher molecular weights are built up of smaller peptides. Such an example is the tobacco mosaic virus which is built up of 2 400 peptides with the molecular weight of 17 500. An other example is haemoglobin having a molecular weight of 66 000 and composed of four peptide chains. Many other examples can be given. It seems that an optimal molecular weight for the peptide chains formed could be anywhere in the vicinity of 17 000—20 000. It is quite possible, in spite of all criticism, that there is a kernel of truth in the old theory of Svedberg [15] that all larger proteins were built up from a subunit of molecular weight 17 500.

There must be many exceptions to this rule. In every case where an exception is suspected the structure must first be investigated so that it is really certain that only one long peptide chain occurs. It can then be expected that the chain has been built up in at least two steps. In the second step the smaller peptides are synthesized together to longer peptides.

The investigations of the author [16] on the biosynthesis of collagen show that this really occurs. The collagen molecule is built up of three chains, every one having a molecular weight of about 120 000. They are thus much longer than could be expected from what has been said above. However, in this case smaller nucleotide peptides of collagen are first synthesized and are then synthesized together to form the peptide chains in another step outside the cell. This necessity for a two step synthesis will support the presumption of an upper limit for the peptide synthesis on the ribosomes. If the structure of the molecule alone was known one would have imagined that an upper limit did not exist.

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