Amino-Acids, Proteins and Proteolytic Enzymes*

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I

APPROXIMATELY twenty-five amino-acids are generally recognised at the present time as constituents of proteins. It is usually assumed that the amino-acids are linked together in the protein molecule by condensation of the carboxyl group of one amino-acid with the amino group of the next with elimination of water to form an amide or peptide linkage; of such linkages the protein molecule contains very large numbers.

It has been calculated that the number of known amino-acids is fully sufficient to account, by variations in the order in which they are combined, for the existence not only of the clearly differentiated fundamental types of protein but also of the enormous number of individual proteins which are required by biological and immunological theory.

Such an arithmetical calculation is, however, no longer satisfying; we desire to know more precisely what is the influence of the nature of the component amino-acids and of the order in which the latter are combined upon the properties of the protein as a whole. One might anticipate, for example, that the action of proteolytic enzymes on a given peptide linkage would be determined by the properties and arrangement of the amino-acids of which the peptide is built up; it is impossible, however, to regard the properties of a protein as a simple summation of those of its constituent amino-acids.

The remarkable stability of the amino-acids is usually ascribed to their zwitterionic nature, from which results their capacity to form internal salts; the immediate neighbourhood of oppositely charged ionised groups must indeed exercise a dominating influence on the behaviour of the amino-acids. It is therefore to be expected that we shall modify the properties of the amino-acids, and in particular their chemical behaviour, if we combine the ionisable groups in peptide linkage and thereby alter their mutual electro-chemical effects.

The outstanding problem of modern protein chemistry is thus twofold in nature: What effect does combination in peptide linkage exercise upon the different amino-acids? And how is the nature of the peptide linkage itself influenced by the character of the amino-acids which take part in its formation? Although work on both aspects of this question is still in an elementary stage, a number of facts have already been revealed which are significant both from a chemical and a biochemical point of view.

In the first place, I should like to show by a few examples that we can reveal a new series of chemical properties of the amino-acids if we convert the latter, by simple acylation of the amino group, into a condition analogous with that in which they exist in peptides. The simplest case is that of acetylation; if we acetylate the amino group of an optically active amino-acid, we find that contact with a small amount of acetic anhydride at the ordinary temperature is sufficient to catalyse the rapid racemisation of the acetylated amino-acid¹. The striking ease with which this racemisation occurs must be ascribed to an intermediate enolisation:

* Substance of three lectures on "The Chemistry of Proteins" delivered at University College Hospital Medical School, London, on January 20, 23 and 24, 1933.

In this reaction, therefore, different parts of the same molecule which are not directly connected with one another, namely, the peptide linkage and the α -hydrogen atom, exhibit a definite mutual influence. A similar mutual influence but in the opposite sense is shown by the complete absence of racemisation when chloroacetylated amino-acids are treated with acetic anhydride under the same conditions. Such remote actions of different parts of the molecule upon one another are of special importance in enzymic processes; their investigation in model experiments makes it possible to expose properties of the amino-acids and peptides which would otherwise remain hidden and to draw conclusions therefrom as to the processes which these compounds may undergo in metabolism.

A particularly surprising example is provided by pyridylacetylphenylalanine, which is a dipeptide closely related to glycylphenylalanine; this compound, when treated with acetic anhydride and pyridine at the ordinary temperature, is converted into acetyldehydrophenylalanine with liberation of pyridine and water:

The phenylalanine is thus dehydrogenated and the liberated hydrogen is employed to hydrogenate the pyridylacetic acid to pyridine and acetic acid². Here, therefore, we have in one half of the molecule the first example of the direct transformation of an amino-acid into a fatty acid, accompanied by simultaneous unsaturation of the amino-acid constituting the other half to give a dehydroamino-acid. According to the theory of Wieland, a dehydrogenation of this character would require the activation of the amino-acid and the presence of an acceptor for the liberated hydrogen; the activation of the phenylalanine is brought about by its combination in peptide linkage with transient formation of a cyclic anhydropeptide whilst the pyridylacetic acid residue serves as hydrogen acceptor.

According to the prevailing view, the biological degradation of an amino-acid to a keto-acid begins with a dehydrogenation, and conversely the biological synthesis of an amino-acid from a keto-acid ends with a hydrogenation. It is possible to combine examples of both these processes in a single experiment³ *in vitro*. The introduction of the dehydrophenylalanyl residue into aspartic acid yields a cyclic anhydride; dissolution of the latter in normal sodium hydroxide causes transference of two hydrogen atoms from the aspartic acid to the dehydrophenylalanine:

whilst in acid solution the reverse transformation may be accomplished. In the first case, therefore, degradation of the aspartic acid has begun and in doing so has completed the synthesis of phenylalanine from its unsaturated precursor. These experiments show that the hydrogenation: dehydrogenation potentials of the individual amino-acids are different; in metabolism there must indeed exist among the amino-acids a constant competition for hydrogen, the outcome of which will vary with circumstances.

By similar facile reactions it is possible to convert serine with elimination of water and cysteine with elimination of hydrogen sulphide into dehydroalanine and thence into pyruvic acid; serine and cysteine are thus brought into genetic relationship with the simple amino-acids and their nitrogen-free transformation products⁴.

Of all the amino-acids, the most widely distributed is arginine, which occupies a special position also by virtue of its guanido group. Arginine can be converted into a peculiarly reactive condition by complete acetylation with simultaneous elimination of water. The triacetylanhydroarginine so formed has a strong tendency, in presence of water and of hydroxy or amino compounds in general, to lose the cyanamide residue, which is itself transferred to the accompanying compound; a mixture of sarcosine with triacetylanhydroarginine, for example, yields creatine together with a derivative of ornithine⁵.

The latter result is of interest in relation to two physiological processes: (1) the creatine synthesis *in vitro* shows how creatine-nitrogen can be derived from two different amino-acids; it is probable that in the course of metabolism creatine is formed in a similar manner from two amino-acids of which one is arginine; (2) the conversion of arginine into ornithine by the enzyme arginase demands that the latter be attached to the arginine molecule in such a manner that the arginine is activated and hydrolysed to ornithine even by cold water; this process is imitated by the ease of hydrolysis of triacetylanhydroarginine to acetyl derivatives of ornithine and of urea.

There remains to be discussed yet another noteworthy transformation of arginine which has long caused confusion in the literature; if arginine be esterified with methyl alcohol, the resulting ester passes spontaneously into ornithine methyl ester and bisguanidovaleric acid:

This reaction must occur in the metabolism of those shell-fish from which Kutscher, Ackermann and Flössner⁶ have isolated arcaine (1:4-diguanidobutane).

The few examples which have been given indicate what an abundance of unsuspected reactions can be elicited from the amino-acids if their zwitterionic character is destroyed by combination in peptide linkage. Almost every amino-acid is affected differently in this respect; conversely, therefore, every amino-acid must have a different influence on the peptide bond.

In order to be able to study this influence on

representative material, it is necessary to prepare different peptide combinations artificially and to test the action of enzymes upon them. To carry this task to its logical conclusion would be a formidable undertaking; fortunately, however, it suffices to prepare peptides containing representative members of the most important types of amino-acids.

Chemists have striven for more than thirty years to discover suitable methods for the synthesis of peptides containing the more complex amino-acids, but until recently only isolated successes have been obtained⁷. Quite lately, however, a general method has been developed⁸, the essential feature being the nature of the acid residue used to block the amino group.

The acid chloride of benzylcarbonic acid is readily obtained by the interaction of benzyl alcohol and phosgene and can be condensed with any amino-acid to yield the corresponding benzylcarbonato derivative. The benzylcarbonato-amino-acids are beautifully crystalline substances which can be easily transformed into their acid chlorides or azides and these in turn can be condensed with other amino-acids to yield benzylcarbonatopeptides. Most important, however, is the fact that the benzylcarbonato residue can be split off without the employment of a hydrolytic agent, so that it can be eliminated from a benzylcarbonatopeptide without danger of scission of the peptide bond. The removal of the benzylcarbonato residue is accomplished by catalytic hydrogenation in presence of palladium black, under which conditions the benzyl residue is eliminated as toluene and the resulting carboxy-amino derivative loses carbon dioxide spontaneously to give the free peptide. The last step of the process proceeds not only with ease but also in most cases almost quantitatively:

A further advantage of the benzylcarbonato method is that it involves no risk of racemisation; with its aid, in fact, the preparation of many optically active peptides, which have hitherto been practically inaccessible, becomes a trivial matter.

A complete picture of the possibilities of the new method of peptide synthesis cannot

be given here, but it is possible to indicate some of the new lines of research which it opens up; in particular it may be of interest to mention certain hitherto unobtainable peptides which have been prepared by the benzylcarbonato method and to describe their behaviour towards enzymes.

As representative peptides we may mention: d-Glutamyl-d-glutamic acid, a substance with three carboxyl groups and one amino group and therefore strongly acidic in character; d-Lysyl-d-glutamic acid, the first example of a dipeptide of a diamino-acid

and an amino-dicarboxylic acid; *d*-Lysyl-*l*-histidine, a dipeptide of two strongly basic amino-acids; *l*-Asparagyl-*l*-tyrosine and *d*-glutamyl-*l*-tyrosine, two closely related dipeptides which, however, differ remarkably in their behaviour towards enzymes (see below); *l*-Tyrosyl-*l*-tyrosine, a dipeptide containing two phenolic groups; *Glycyl*-*l*-proline and *d*-alanyl-*l*-proline, which occupy a distinctive position owing to the nature of the peptide linkage:—

For comparison with these proline peptides another dipeptide in which the nitrogen of the peptide linkage is tertiary in character, namely, glycylsarcosine, has been prepared.

In the following table, the behaviour of these dipeptides is shown towards dipeptidase, towards aminopolypeptidase, and towards trypsin (that is, a mixture of proteinase and carboxypolypeptidase):

	Dipeptidase.	Amino-polypeptidase.	Trypsin (Carboxy-polypeptidase + proteinase).
<i>d</i> -Glutamyl- <i>d</i> -glutamic acid	++	—	—
<i>d</i> -Lysyl- <i>d</i> -glutamic acid	++	—	—
<i>d</i> -Lysylglycine	++	—	—
<i>d</i> -Lysyl- <i>l</i> -histidine	++	—	—
<i>l</i> -Asparagyl- <i>l</i> -tyrosine	—	—	—
<i>d</i> -Glutamyl- <i>l</i> -tyrosine	++	—	—
<i>l</i> -Tyrosyl- <i>l</i> -tyrosine	++	—	+
<i>d</i> -Tyrosyl- <i>d</i> -arginine	++	—	+
Glycyl- <i>l</i> -proline	—	++	—
<i>d</i> -Alanyl- <i>l</i> -proline	—	++	—
Glycylsarcosine	—	0	—

With glutamylglutamic acid the result is as one would expect; this dipeptide, in spite of its strongly acidic character, is hydrolysed by dipeptidase after suitable buffering.

Lysylglutamic acid on the other hand is attacked only with difficulty by dipeptidase; lysylglycine is readily hydrolysed by this enzyme and the same is true of the strongly basic lysylhistidine. These

examples show that the preponderance of acidic or of basic groups does not interfere with dipeptidase action, since the most strongly acidic and most strongly basic dipeptides are both attacked by the enzyme. Surprising differences of behaviour between closely related compounds are exemplified by asparagyl- and glutamyl-tyrosine, of which the first is resistant to all intestinal proteolytic enzymes whilst the second is readily hydrolysed by dipeptidase.

l-Tyrosyl-*l*-tyrosine presents an anomaly inasmuch as it is hydrolysed not only by dipeptidase but also by trypsin, whilst *d*-tyrosyl-*d*-arginine is actually not affected by dipeptidase but is hydrolysed by carboxypolypeptidase.

The behaviour of the proline peptides towards proteolytic enzymes is especially illuminating. Neither glycylproline nor alanylproline is attacked by dipeptidase whilst both are hydrolysed by aminopolypeptidase; here therefore we have two dipeptides for which dipeptidase is not the appropriate enzyme, and it becomes clear that the accepted definitions of dipeptidase and aminopolypeptidase are unsatisfactory. More important still is the conclusion which emerges from the experiments with the proline peptides that 'dipeptidase' can only attack a normal peptide linkage (—CONH—), whilst at least one constituent of 'aminopolypeptidase' can hydrolyse a peptide linkage in which the nitrogen atom is tertiary. This view is confirmed by the fact that glycylsarcosine is also resistant towards dipeptidase.

¹ Bergmann and Zervas, *Biochem. Z.*, **203**, 280; 1928.

² Bergmann, Zervas and Lebrecht, *Ber. deutsch. chem. Ges.*, **64**, 2315; 1931.

³ Bergmann and Ensslin, *Z. physiol. Chem.*, **174**, 76; 1928; Bergmann and Stern, *Liebigs Ann.*, **448**, 20; 1926; Bergmann, Kann and Miekeley, *Liebigs Ann.*, **449**, 137; 1926; Bergmann and Miekeley, *Liebigs Ann.*, **458**, 40; 1927.

⁴ Bergmann and Delis, *Liebigs Ann.*, **458**, 76; 1927; Bergmann and Stather, *Z. physiol. Chem.*, **152**, 190; 1926.

⁵ Bergmann and Köster, *Z. physiol. Chem.*, **159**, 179; 1926; Bergmann and Zervas, *Z. physiol. Chem.*, **172**, 277; 1927; **173**, 80; 1928.

⁶ Zervas and Bergmann, *Ber. deutsch. chem. Ges.*, **61**, 1195; 1928; also *Z. physiol. Chem.*, **201**, 208; 1931; Kutscher, Ackermann, Flössner and Hoppe-Seyler, *Z. physiol. Chem.*, **199**, 273 and 277; 1931.

⁷ Bergmann, Stern and Witte, *Liebigs Ann.*, **449**, 277; 1926; Bergmann and Köster, *Z. physiol. Chem.*, **167**, 92; 1927; Bergmann, Zervas and du Vigneaud, *Ber. deutsch. chem. Ges.*, **62**, 1905; 1929.

⁸ Bergmann and Zervas, *Ber. deutsch. chem. Ges.*, **65**, 1192; 1932; Bergmann, Zervas and Greenstein, *Ber. deutsch. chem. Ges.*, **65**, 1692, 1932; Bergmann, Zervas, Schleich and Leinert, *Z. physiol. Chem.*, **212**, 72; 1932.

West Regional Broadcasting Station in Great Britain

IT will be remembered that a few years ago the British Broadcasting Corporation engineers evolved what is known as the 'regional scheme' for covering Great Britain with an adequate broadcasting service providing alternative programmes of approximately the same strength. According to this scheme, each part of the country is catered for by a twin-wave transmitting station radiating both a national broadcasting programme and a regional programme specially arranged for the area under consideration. The fourth station of this type is now practically complete and experimental programme transmissions were commenced on April 24. The following details of this West Regional Station are taken from an article in *World Radio* of April 21.

The station has been built near the Somerset coast at Washford Cross, two miles from Watchet and six miles east of Minehead. The site was chosen as being suitable for the provision of a good service to the important centres of population in the west of England and to South Wales in addition. The design of the station follows closely the general arrangement of the regional stations, and includes some of

the modifications recently introduced at the Scottish Regional Station. Each of the two aerials employed is of the 'umbrella' type supported by an insulated lattice steel mast 500 ft. high. These two masts are illuminated at night by aircraft warning lights, which are fed through filter circuits to prevent the flow of high-frequency current in the lamp leads.

The whole of the electrical power required is generated locally by Diesel engine plant and sufficient fuel oil is stored for three months operation. For the various electrical supplies required, three of each type of motor-generator set are installed; one of these is in use on each transmitter, while one is spare. The national and regional transmitters are similar and of a standardised type of construction comprising five separate units. Modulation is carried out at an early stage following the drive oscillator. Two stages of high-power amplification follow, while the fifth unit contains the tuning and coupling circuits for the final power stage. In each stage it is possible to switch in a spare valve without interrupting the power supply. A studio for testing purposes is fitted up in the station buildings, although the programmes will normally emanate from the