Energetics of Peptide Formation

HIGH-MOLECULAR polypeptides containing only one kind of amino-acid are prepared by polymerization of N-carboxylic-α-amino-acid anhydrides[1] (α-amino-acid-NCA). The determination of the heat of combustion of these compounds and of the corresponding amino-acids offers for the first time the possibility of estimating quantitatively the energetics of formation of high-molecular peptide.

Bomb calorimetric measurements were carried out with *d,l*-phenylalanine and sarcosine derivatives. The heats of combustion (cal./gm.) and combustion enthalpies (kcal./mole) of these compounds are given in Table 1.

Table 1. HEATS OF COMBUSTION AND COMBUSTION ENTHALPIES

Compound	Heat of combustion (cal./gm.)	Combustion enthalpy (kcal./mole)
d,l-Phenylalanine	6,727·5	−1,112·3
d,l-Phenylalanine-N-carboxylic acid anhydride	5,835·8	−1,116·1
d,l-Phenylalanine polypeptide, benzene soluble	7,503·2	−1,105·2
d,l-Phenylalanine polypeptide, benzene insoluble	7,479·1	−1,101·7
Sarcosine	4,484·3	− 400·0
Sarcosine-N-carboxylic acid anhydride	3,509·6	− 403·8
Sarcosine polypeptide	5,626·1	− 400·3

Molar combustion enthalpies of polypeptides are expressed in terms of chain units neglecting the end-groups. From the combustion enthalpies as given in Table 1, reaction enthalpies were calculated and are shown in Table 2.

Table 2. REACTION ENTHALPIES

Reaction	Reaction enthalpy (kcal./mole)
Phenylalanine-N-carboxylic acid anhydride (s) → polypeptide, benzene soluble (s) + carbon dioxide (g)	−10·9
Polypeptide, benzene soluble (s) → polypeptide, benzene insoluble (s)	− 3·5
Phenylalanine (s) → polypeptide, benzene soluble (s) + water (l)	− 7·1
Sarcosine-N-carboxylic acid anhydride (s) → polypeptide (s) + carbon dioxide (g)	− 3·5
Sarcosine (s) → polypeptide (s) + water (l)	+ 0·3

The formation of high-molecular polypeptides by polymerization of N-carboxylic acid anhydrides is an exothermic reaction as expected. The transformation of the benzene-soluble form of phenylalanine polypeptide into the insoluble one is also exothermic.

Also given in Table 2 is the reaction enthalpy for the hypothetical condensation of the amino-acids to the corresponding polypeptides. With phenylalanine, this hypothetical reaction is exothermic, whereas with sarcosine it is nearly athermic. The last two results are in striking contrast with the hitherto known endothermic reactions of glycine yielding glycylglycine, diglycylglycine and triglycylglycine, and glycine and leucine yielding glycylleucine[2]. The substitution of one hydrogen atom in the CH_2- or NH_2-group of glycine by different groups obviously has an important influence on the energetics of peptide formation.

It therefore seems likely that, in energetically favourable cases, the condensation of amino-acids will be spontaneous; in other words, it will not need to be coupled with another energy-producing process.

A full account of this investigation will be published in the near future in *Monatshefte für Chemie*.

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A New Method for the Detection of Peptides and Similar Compounds on Paper Chromatograms

SINCE the first introduction[1] of paper chromatography for the separation and identification of amino-acids and peptides, ninhydrin has been used almost exclusively as the spraying reagent to reveal the positions of the spots, although some use has been made of ultra-violet fluorescence[2] and of spraying with potassium permanganate[3] and specific reagents for individual amino-acids[4]. Ninhydrin, however, has two serious limitations: it does not react with cyclic peptides or with acylated amino-acids or peptides, while with peptides, since only the terminal amino-group enters into the reaction, the colour developed with a given amount of material decreases with increasing molecular weight.

We have found that peptides of all kinds, including proteins, diketopiperazine and acylated amino-acids and peptides, can be very easily detected by chlorination and subsequent spraying with starch–potassium iodide, the colour being, of course, due to the liberation of iodine by the N-chloro-peptide formed in the chlorination stage:

$$\text{—CO—NH—} \xrightarrow{Cl_2} \text{—CO—NCl—} \xrightarrow{KI} \text{—CO—NH—} + KCl + \tfrac{1}{2}I_2.$$

The chromatogram is run and the solvent removed, as completely as possible, in the usual manner; thorough removal of the solvent is essential if an over-intense 'background' coloration is to be avoided, and it is generally desirable first to heat the paper to 60° for two hours and then to hang it in air overnight, followed by a final heating to 60° for 30 min. The paper is then loosely rolled and placed in an atmosphere of gaseous chlorine for 10 min., conveniently in a loosely corked, dry gas-jar. The paper is then removed and hung in a current of air at room temperature for 30 min. or until a trial portion shows little or no 'background' on spraying with the starch–iodide reagent. The chromatogram is finally sprayed lightly and evenly with 1 per cent starch – 1 per cent potassium iodide solution. The compounds are at once clearly revealed as intense blue-black spots on a faint blue background; the spots fade to brown in the course of a few hours and fade completely in a few days; they should, therefore, be marked or photographed as soon as possible after the final spraying. There is no advantage in replacing the starch by sodium starch glycollate[5] or polyvinyl alcohol[6]. If desired, the chlorine–starch–iodide procedure can be applied successfully to papers which have already been sprayed with ninhydrin.