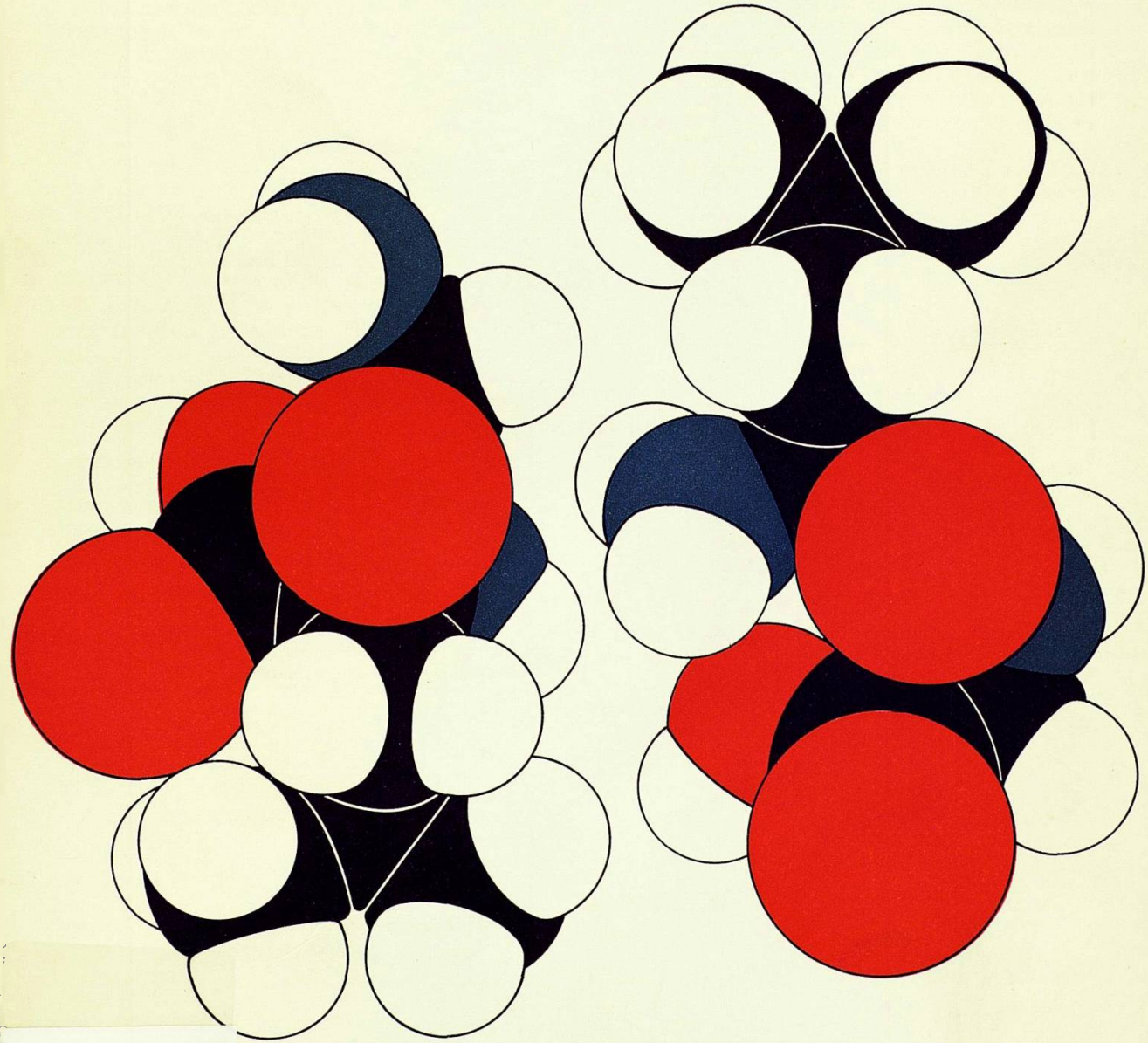
N-terminal analysis of a dipeptide

A **UNILEVER**
LABORATORY
EXPERIMENT

NUMBER 8



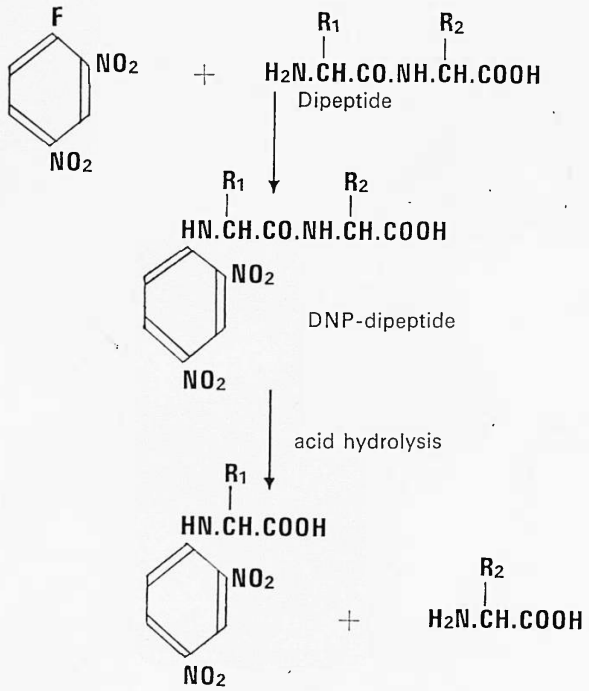
Molecular dipeptide models of glycyl-DL-leucine and DL-leucyl-glycine

N-terminal analysis of a dipeptide

This experiment was devised by St Paul's School in collaboration with Unilever Research

If the two constituent amino acids of a dipeptide are known a unique amino acid sequence can be assigned to the dipeptide provided the nature of the amino acid with a free amino group can be determined. This process is referred to as N-terminal analysis.

One of the best established techniques for this purpose is the 1-fluoro 2,4 dinitrophenylation procedure developed by Sanger during his studies on the structure of the protein hormone insulin. In this method 1-fluoro 2,4 dinitrobenzene (FDNB) is reacted with the free amino group of the peptide or protein under mildly alkaline conditions, to yield a dinitrophenyl (DNP) peptide or protein. After acid hydrolysis the N-terminal amino acid is recovered as the yellow DNP-amino acid and can be characterized by paper chromatography.



DNP-amino acid

In the experiment to be described, two dipeptides are used which contain the same two amino acids but in different sequence. Hence normal amino acid analysis could not differentiate them, but N-terminal analysis can.

This method of Sanger's, with care, can be made quantitative and used to estimate the molar ratio of N-terminal amino acid to protein, thus permitting the determination of the number of polypeptide chains in each molecule of the protein.

Precautions

FDNB has vesicant properties and a pair of thin rubber or protective polythene gloves should be worn while handling it. FDNB reacts with ammonia, and is then innocuous. Therefore all glassware used to handle this chemical should be placed in ammonia solution before washing.

The yield of DNP-amino acid will be improved if, during the procedure, materials are stored in the dark, as DNP-amino acids undergo photodecomposition.

Apparatus

2 ignition tubes
2 soda-glass test tubes, drawn out to narrow necks to facilitate sealing off
0.1 ml, 1 ml and 2 ml pipettes + safety attachment for pipetting FDNB
Separating funnels (2 × 50 ml; 1 × 250 ml or 1 × 500 ml)
Boiling tube (3.5 cm × 15 cm) with split cork
250 ml beaker to act as a water bath
0-110°C thermometer
2 × 4 cm diam. crucibles
Whatman No. 1 chromatography paper (3 cm wide roll)
Drawn-out glass tubing applicators

Chemicals

FDNB
Glycyl-DL leucine
DL-leucyl-glycine*
0.4M potassium bicarbonate solution
Industrial methylated spirits
Concentrated hydrochloric acid
Di-ethyl ether
Acetone
n-Butanol (butan-1-ol).

*FDNB is available from BDH Ltd; dipeptides are available from Koch-Light Laboratories Ltd, Colnbrook, Beds.

Procedure

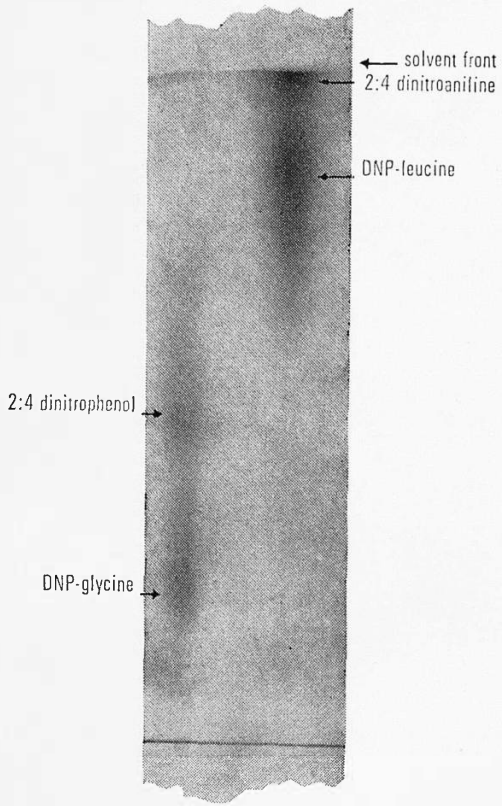
The two peptides should be treated in parallel so that the results can be compared. Approximate time required: 2 double practical periods.

Incubation

10 mg of each peptide is dissolved in 2 ml of 0.4M potassium bicarbonate solution in an ignition tube and 1 ml of a 2% (v/v) solution of FDNB in industrial spirit added. This is an approximately threefold excess of FDNB. The tubes are transferred to a water bath and the contents incubated at 40°C for 30 minutes.

Hydrolysis

The incubated mixtures are transferred to small separating funnels and washed twice with a small quantity of ether (3-5 ml) to remove excess FDNB. The lower aqueous layer is run off each time, and the residual ether layer is treated with ammonia solution and discarded. The aqueous phases containing the DNP peptides, are finally transferred to soft-glass test tubes (previously drawn out to narrow necks) and approximately equal volumes of concentrated hydrochloric acid added. The tubes are then sealed by drawing off in a flame, wrapped in wire gauze, and placed in an oven at 105°C for 5-6 hours for hydrolysis of the peptides to occur.



Extraction and Chromatography of DNP-amino acids

The test tubes are allowed to cool and opened. Their contents are transferred to small separating funnels and extracted twice with a small quantity of ether (3-5 ml). This time the ether layers contain the DNP-amino acids and these are transferred to crucibles and allowed to evaporate to dryness.

The chromatograms can be run in boiling tubes, the solvent being placed in the bottom and the paper held by the split cork. For chromatography, the DNP-amino acids are dissolved in a small quantity of acetone and applied to a suitable narrow strip of Whatman No 1. paper using finely drawn out glass tubes. The solvent system is water saturated n-butanol, which can be prepared using the larger separating funnel.

Results

Great care must be taken not to overload the chromatograms otherwise considerable 'tailing' of the spots will occur. The DNP-amino acids appear on the chromatograms as yellow spots, which fade if left exposed to light. DNP glycine has an R_f value around 0.30-0.35 and DNP-leucine around 0.75. Among the artefacts which may be seen are 2 : 4 dinitroaniline (R_f=0.95) and 2 : 4 dinitrophenol (R_f around 0.55-0.60). These artefacts can be faded to some extent by exposure to hydrochloric acid fumes.

Although the above R_f values may not be very reproducible except under carefully controlled conditions, comparison of the results obtained for the two peptides shows a clear distinction (see chromatogram).

Unilever

Unilever is an international company with head offices in London and Rotterdam.
Unilever interests include:
Washing products
Toilet preparations
Food
Edible fats
Oil milling and animal foods
Chemicals
Perfumes and flavours
Plantations

The precise form of its protein structure is known to influence the texture of food. All techniques of protein analysis are of importance to Unilever with its wide interests in processed foods and animal feedingstuffs.

Published by Information Division
Unilever 1967
Reprinted, 1969
Reprinted, 1973
Designed by Paul Reeves L.S.I.A
Printed in England by Mackay, Chatham

Unilever Laboratory Experiment
Number 8
Copies of this free leaflet may be obtained by science teachers from
Unilever Education Section
Unilever House,
London, EC4P 4BQ