A CONVENIENT METHOD FOR PREPARATION OF ^{14}C-LABELLED BASIC NUCLEAR PROTEIN - HISTONE.

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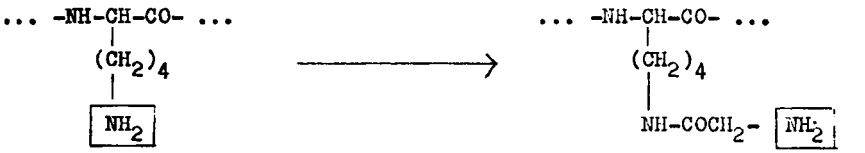
Preparation of proteins labelled with radioisotopes is still a topical problem. The iodination of some proteins with iodine isotopes may serve as an example. Another method consists in the acetylation (or generally acylation) of free amino groups, for which both ^{14}C and ^{3}H may be used. Holt and co-workers (1) acylated wool with p-nitrophenylesters of ^{14}C-labelled fatty acids and observed that the acylation took place predominantly at ϵ-amino groups of lysine residues. Using activated esters Carpenter (2) described the synthesis of non-radioactive diaminoacyl- and triaminoacyl-insulines which still possessed 40-50 % of the biological activity of insulin itself. The use of N-carboxy anhydrides of amino acids (e.g. (3)) represents another way of introducing aminoacyl residues to a protein molecule. However, this latter method is not very suitable for the preparation of labelled proteins as it requires excess radioactive N-carboxyanhydride and two or more radioactive aminoacyl residues can bind to the free amino group.

In the case of basic proteins, e.g. histone, it is convenient to carry out substitution of free hydrogen atoms of the ϵ-amino groups of lysine by labelled glycine:



The convenience of this method consists in the fact that the original basic character of the molecule remains intact, while a small change in the length of the side chain might not be deleterious for the biological properties.

The above described procedure was successfully used (4) for labelling basic peptide antibiotics (polymyxin B, colistin) by means of Woodward's reagent (i.e. 2-ethyl-5-phenylisoxazolium-3'-sulphonate) (5).

Histone labelled with radioisotopes in its amino acids was prepared so far only biosynthetically. In addition preparations with a very low specific radioactivity (6), as for example, the lysyl-^{14}C-histone prepared from Ehrlich ascites tumor after *in vitro* labelling with lysine-^{14}C, had a specific activity of 100-500 dpm/μmol lysine.

The method used by us enables to synthesize preparations with much higher specific activities, and, at the same time, the radioactive material is utilized more economically.

Experimental

Glycyl-^{14}C-histone (arginine-rich)

To a solution of o-nitrobenzensulphenylglycine-^{14}C (U) (set free from its dicyclohexylammonium salt: 31.8 mg; 77.6 μmol of radioactivity 152 μCi) in 1.55 ml acetonitrile, 0.31 ml of a solution of N-ethylpiperidine (containing 8.77 mg, i.e. 76.2 μmol) in acetonitrile and 2-ethyl-5-phenylisoxazolium-3'-sulphonate (19.9 mg; 78.6 μmol) were added and the mixture was shaken until the reagent dissolved (30 min.). The obtained solution was added to a solution of arginine-rich histone (obtained from calf thymus (7);

97.5 mg) in 6.2 ml ice-water and 0.78 ml of 0.1 N NaOH. The reaction mixture was stirred for 22 hours at 0°C and then freeze-dried in two 50 ml flasks. Three ml of ethanol containing 6 N HCl (9:1, v/v) were added with cooling to each flask and the mixture was shaken for 20 minutes. After centrifugation the sediment was triturated twice with ethanol containing 1.25 N HCl (8:2, v/v). The product was then triturated with acetone (3 ml) with cooling and was left to stand for 2 hours. It was centrifugated after that interval, washed four times with 0.6 ml of acetone and dried in a vacuum dessicator. The yield was 67.6 mg, the radiochemical yield was 45.5 μCi (30 %), i.e. specific activity 0.67 μCi/mg or 1.3×10^6 dpm/μmol of lysine (according to the analysis of amino acids). On checking the purity by electrophoresis it was found that the product is homogeneous and does not contain either radioactive glycine or other radioactive impurities.

From the amino acid analysis of the starting histone and of the "glycylated" histone it can be deduced that about 1/3 of the ϵ-amino groups of lysine molecules are aminoacylated with radioactive ^{14}C-glycyl residues.

Biological properties.

Arginine-rich glycyl-^{14}C-histone was successfully used for studying the transport of this compound inside the cells of Escherichia coli ML 30 (8). The biological activity of this modified histone was completely identical with that of the original one, when studying the inhibitory effect of histone on the inducible synthesis of β-galactosidase in Escherichia coli during the logarithmic phase of growth and the stimulatory effect in the stationary phase of growth.

It can be assumed that the convenient method of preparation described here will also be useful with other basic proteins.

References

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