RADIOACTIVE LABELING OF PROTEIN *IN VITRO* BY ACETYLATION WITH ^{14}C-*N*-(ACETOXY)-SUCCINIMIDE. A NEW METHOD FOR QUANTITATIVE DETERMINATION OF PROTEIN IN THE PRESENCE OF POLYNUCLEOTIDES

V.P. DEMUSHKIN and E.I. BUDOWSKY
Shemyakin Institute for Chemistry of Natural Products, USSR Academy of Sciences, Moscow, USSR

Received 30 December 1971
Revised version received 9 February 1972

1. Introduction

Radioactive labeling is a useful method for tracing minor amounts of protein. Chemical methods of labeling, such as iodination [1] or reductive alkylation [2], are convenient but sometimes inapplicable since they cannot be used when protein is to be determined in the presence of nucleic acids. It was anticipated that reaction of organic acid *N*-hydroxysuccinimide esters would be more useful because under appropriate conditions they readily acylate amino acid amino groups leaving nucleic acids intact [3]. The present communication describes a method of quantitative determination of protein based on the introduction of a radioactive label by a reaction of this type.

2. Materials and methods

^{14}C-Acetic acid (6 Ci/mole) was a commercial preparation ("Isotope", USSR), human albumin, ribonuclease and DNA of chick erythrocytes were from "Reanal" (Hungary); tRNA of *E. coli* was isolated according to Gutcho [4]. The synthesis of ^{14}C-*N*-(acetoxy)-succinimide was performed essentially according to Gillam et al. [5]. 3.5 ml of *N*-hydroxysuccinimide in aqueous-dioxan solution (57 mg/ml, 1.75 mmoles; water:dioxan, 1:4) were added to 0.96 ml (1.7 mmoles) of ^{14}C-acetic acid followed by 3.6 ml of dicyclohexylcarbodiimide in dioxan (103 mg/ml, 1.80 mmoles). The reaction mixture was stirred for 4 hr at 25°, the precipitate removed by filtration and washed with 20 ml of dioxan. The combined filtrates were evaporated to dryness and the residue four times evaporated from dry dioxan solution (10 ml). Yield was 80% on a radioactivity basis. The residue was dissolved in dry dioxan to adjust the concentration of ^{14}C-*N*-(acetoxy)-succinimide to 0.04 M and the solution stored at −5° in sealed tubes. The preparation can be stored under these conditions during at least a year.

The introduction of a radioactive label into proteins was performed as follows: 0.05 ml of 0.04 M ^{14}C-*N*-(acetoxy)-succinimide dioxan solution was added to 0.05 ml of protein solution (up to 10 μg) at 0° followed by 0.05 ml of 0.1 M phosphate buffer, pH 8.0. The mixture was kept at 0° for 10 min and either diluted to 0.5 ml and dialyzed, or applied to a paper disk and the latter treated with trichloroacetic acid to fix protein [6].

The radioactivity was measured with gas-flow counter (efficiency for ^{14}C was 60%). The reaction with nucleic acids was studied under the conditions outlined above.

3. Results and discussion

The reaction of ^{14}C-*N*-(acetoxy)-succinimide with

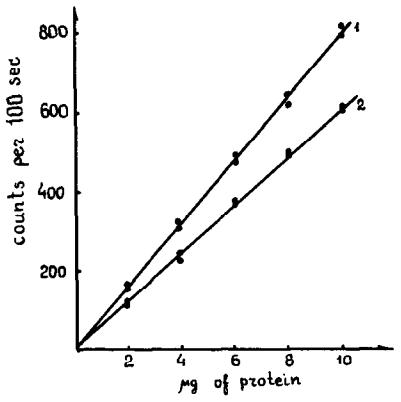

Fig. 1. Dependence of the incorporation of ^{14}C-acetyl groupings into proteins on the amount of protein in the reaction mixture. 1: Ribonuclease; 2: Human albumin.

protein is complete within 10 min and addition of a fresh portion of reagent or further incubation does not lead to an increase of the extent of acylation. Under these conditions, incorporation of radioactivity into human albumin is 6×10^5 counts per 100 sec, into ribonuclease, 8×10^5 counts per 100 sec, per 1 mg of protein. It is seen in fig. 1 that the incorporation of radioactivity is directly proportional to the amount of proteins. Hence, acetylation with labeled *N*-(acetoxy)-succinimide can be used as a highly sensitive method of protein determination. Obviously, the sensitivity of the method depends on the number of exposed amino groups in given protein.

^{14}C-*N*-(acetoxy)-succinimide practically does not react with nucleic acids under the conditions for acylating proteins. It was found that incorporation into polynucleotides does not exceed $3-5 \times 10^{-3}$ moles of acetyl groupings per mole of nucleoside residues. Further incubation or addition of fresh reagent does not increase this incorporation. Hence, the incorporation found seems to be due to acetylation of trace admixture of proteins rather than to acylation of nucleoside residues. The results obtained on the phage MS2 are in accord with the above data. After destruction of the acetylated phage particles by the action of urea and sodium dodecylsulfate and gel-filtration of the mixture on Sephadex G-200, phage RNA contains ca. 3×10^{-3} μmole of acetyl groups per μmole of nucleoside residues (10^2 counts per 1 mg), while incorporation of radioactivity into phage protein is 4×10^5 counts per 100 sec per 1 mg of protein (details of this experiment will be described elsewhere).

Hence, reaction with ^{14}C-*N*-(acetoxy)-succinimide makes it possible to introduce a radioactive label into proteins *in vitro* under extremely mild conditions and to determine proteins quantitatively in the presence of nucleic acids. It seems also that the reaction might be a useful tool with which to study functional properties and structures of proteins.

Acknowledgement

The authors are thankful to M.I. Ivanov for skillful assistance.

References

[1] R.W. Helmkamp, M.A. Contreras and W.F. Bale, Int. J. Appl. Radiat. Isotopes 18 (1967) 737.
[2] R.H. Riese and G.B. Means, J. Biol. Chem. 246 (1971) 831.
[3] V.P. Demushkin, O.D. Nelidova and E.I. Budowsky, Molekul. Biol. 5 (1971) 858.
[4] S. Gutsho, Biochim. Biophys. Acta 157 (1968) 76.
[5] I. Gillam, D. Blew and R.C. Warrington, Biochemistry 7 (1968) 3459.
[6] S. Nishimura and G.D. Novelli, Biochim. Biophys. Acta 80 (1964) 547.