Short Communications

The Use of Radiolabelled T₁ Ribonuclease to Monitor the Efficiency of Edman Degradations

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A simple radiolabel tracer method is presented for monitoring the efficiency of the first ten cycles of an Edman degradation. This method consists of adding a very small amount of radiolabelled *S*-carboxymethylated T₁ ribonuclease to the protein to be sequenced, and counting the radioactivity of a small sample from each of the degradative cycles.

An Edman degradation may fail to produce the expected yields of amino acid derivatives for one of two reasons: either (1) the mechanical manipulations or chemical reactions of the degradation procedure do not work, or (2) the sample protein is intrinsically unable to be degraded (for example, because it has no free α amino group). We report here a simple radioactive tracer method for monitoring the efficiency of the degradation procedure without interfering with the sequence analysis of the non-radioactive sample protein. When a degradation fails to produce the expected yields, this method can show immediately whether or not the first of these two factors is responsible, and thus save considerable effort in finding the cause of failure.

Materials and methods

S-[³H]Carboxymethylated T₁ ribonuclease was prepared as follows. A 2mg sample of T₁ ribonuclease from *Aspergillus oryzae* (an enzymically inactive preparation given by Worthington Biochemicals, Freehold, N.J., U.S.A.) was reduced with 10 μ mol of dithiothreitol in 2ml of 7M-guanidinium chloride–0.2M-Tris, adjusted to pH8.6 with HCl, for 90min at 37°C. Then 16 μ mol of iodo[³H]acetic acid (80% of the total thiol groups present) containing 1mCi of ³H was added. After 30min at room temperature, 22 μ mol of non-radioactive iodoacetic acid was added and the solution incubated for an additional 30 min to complete the carboxymethylation of the protein. The protein was freed of unbound radioactivity and non-volatile salts by passage through a column of Sephadex G-15 equilibrated and eluted with 0.2M-NH₄HCO₃. The final solution was diluted to a concentration of about 5 $\times$ 10⁷ d.p.m./ml. Approx. 5% of the added ³H was bound to the protein by this procedure. ¹⁴C-labelled T₁ ribonuclease can be

prepared by an analogous procedure with iodo-[¹⁴C]acetic acid.

To illustrate the use of the ³H-labelled ribonuclease in an Edman degradation, we added 4.54 $\times$ 10⁵ d.p.m. (about 0.4nmol) of this tracer to 100nmol of whale myoglobin (Sigma Chemical Co., St. Louis, Mo., U.S.A.) and sequenced the mixture with an Edman-Begg sequenator (Illitron Division, Illinois Tool Works, Chicago, Ill., U.S.A.), by using procedures and chemicals described by Smithies *et al.* (1971). A 1.3% sample of the amino acid thiazolinone-containing extract from each step of the degradation was mixed with 10ml of Bray's (1960) solution and counted for radioactivity in a scintillation counter. The remainders of the extracts were evaporated and hydrolysed with HI to regenerate free amino acids, which were identified and determined quantitatively by amino acid analysis (Smithies *et al.*, 1971).

Results and discussion

Since the ³H-labelled ribonuclease has radioactive *S*-carboxymethylcysteine residues at positions 2, 6 and 10 (Takahashi, 1971), successful Edman degradation will result in radioactivity in the extracts from the corresponding steps. (The protein also contains *S*-[³H]carboxymethylcysteine at position 103, but this residue is ordinarily too far from the *N*-terminus to be accessible to Edman degradation.)

Table 1 shows the yields of radioactivity resulting from the degradation of the ³H-labelled ribonuclease as compared with the yields of leucine from the simultaneously degraded non-radioactive myoglobin. Radioactivity from *S*-[³H]carboxymethylated T₁ ribonuclease was obtained in high yields at steps 2, 6 and 10, as expected. Leucine from myoglobin was obtained in high yields at steps 2, 9 and 11, which are positions of leucine residues in the myoglobin

Table 1. Yields of radioactivity and leucine from an Edman degradation of a mixture of *S*-[^3H]carboxymethylated T_1 Ribonuclease

The theoretical yields are the amounts of radioactivity or leucine expected if a single *S*-carboxymethylcysteine or leucine residue was liberated from 100% of the ^3H -labelled enzyme or myoglobin molecules respectively.

Step of degradation	Yield (% of theoretical)	
	Radioactivity from <i>S</i> -[^3H]carboxymethylated T_1 ribonuclease	Leucine from myoglobin
1	3.8	14.4
2	61.5	79.0
3	6.6	6.0
4	2.5	1.0
5	2.3	11.7
6	49.0	1.7
7	9.7	1.6
8	3.3	3.0
9	2.6	40.9
10	38.9	19.7
11	8.9	46.0
12	3.4	22.7

sequence (Edmundson, 1965). The other amino acids from myoglobin were also obtained in high yields at the expected positions (results not shown).

The ^3H -labelled ribonuclease did not interfere with the chemical identification of the amino acid derivatives from myoglobin, since so little mass of this tracer was added. These data indicate that both ^3H -labelled ribonuclease and myoglobin were efficiently degraded in this experiment.

In summary, if *S*-[^3H]carboxymethylated T_1 ribonuclease is added to a sample protein, the yields of radioactivity show whether or not the mechanical manipulations and chemical reactions of the degradation work correctly. If low yields of amino acids are obtained from the sample protein despite good yields of radioactivity from ^3H -labelled ribonuclease it is very likely that the sample protein is intrinsically unable to be efficiently degraded by the procedure used.

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