Application of FTC-Method to Amino Acid Sequence Analysis of the Peptidyl Factor in *Saccharomyces cerevisiae*

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α Substance- I_A is a peptidyl factor which induces sexual agglutination prior to the mating reaction in the heterothallic strains of the yeast, *Saccharomyces cerevisiae*. Quite recently, Sakurai *et al.*¹⁾ elucidated the amino acid sequence of the peptide by the micro dansyl-Edman method as illustrated below, though they were unable to differentiate glutamic acid (Glu) and glutamine (Gln).

H-His-Trp-Leu-Glx-Leu-Lys-Pro-Gly-
Glx-Pro-Met-Tyr-OH

In 1976, Muramoto *et al.*²⁾ reported a practical and highly sensitive manual, named FTC-method, for the sequence determination of peptides by the combined use of fluoresceiniso-thiocyanate (FITC) and phenylisothiocyanate (PITC). Here we applied the method to reconfirm the structure of α substance- I_A as well as to differentiate Glu and Gln in the peptide. As the result, the sequence of ten amino acids from the *N*-terminus was successfully confirmed as the same as reported earlier and, further, the both Glxs in the above formula were established as Glns.

In this experiment the FTC-method was applied to a very small amount of the peptidyl factor by two different ways:

(A) The procedure was similar to that in the previous report.²⁾ The peptide (67 nmoles) was transferred to a reaction tube, to which was added 50 μ l of dimethylallylamine buffer (pH 9.5) containing 98 μ g (230 nmoles) of

FITC. The reaction mixture was allowed to stand for 30 min at 45°C under nitrogen atmosphere with occasional vortexing. Then, 1 μ l (8 μ moles) of PITC was added to the mixture to react with the peptide which had been uncoupled with FITC. The mixture was kept at 45°C for an additional 30 min under nitrogen atmosphere.

After completion of the coupling reaction, the mixture was shaken with 50 μ l of benzene-ethylacetate (1:1, v/v) to remove excess FITC and PITC. This manipulation was repeated thrice. The aqueous phase was frozen and dried in a vacuum desiccator (kept at 40°C). The solid residue was rinsed once with 50 μ l of 1-chlorobutane (containing 1,4-butanedithiol at a ratio of 30 μ l/liter) and evaporated again *in vacuo*. Then, the solid was treated with 30 μ l of trifluoroacetic acid for 20 min at 40°C under nitrogen atmosphere. After removal of trifluoroacetic acid *in vacuo*, 50 μ l of ethylacetate was added to the test tube to extract the FITC-derivative of amino acid. The ethylacetate phase was transferred to another test tube, from which the solvent was evaporated. To the residue was added 30 μ l of 50% aqueous trifluoroacetic acid, and the mixture was heated for 5 min at 75°C under nitrogen atmosphere. Then, trifluoroacetic acid and water were removed *in vacuo* to give the FTH-amino acid. The residue of ethylacetate extraction was dried *in vacuo* and submitted to the next cycle of the sequence analysis.

(B) In another run, the C-terminal group of the peptide was modified beforehand by the method of Foster *et al.*³⁾ to prevent the extraction loss of fragmental peptides. The original peptide (25 nmoles) was treated with equivalent moles of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and 7-amino-1,5-naphthalenedisulfonic acid which had been dissolved in 50 μ l of water and adjusted pH 4.0 with 0.01 N HCl. After stirring for 4 hr at room temperature, the reaction mixture was diluted with 100 μ l of 10% acetic acid and freeze-dried. The modified peptide thus obtained was applied to the FTC-method in the same way as described in (A).

FIG. 1. Chromatograms of FTH-amino Acids on Micropolyamide Thin-layer Plates Obtained at Various Steps of the Edman Degradation (The numeral at the corner of each chromatogram is the cycle number of degradation.).

Solvent: (1) acetone-benzene-acetic acid (4: 2: 1, v/v) (2) water-pyridine-acetic acid (10: 8: 1, v/v)
Areas: (black) dominant spot of FTH-amino acid; (dotted outlines) trace spots of FTH-amino acids or other contaminants.

The FTH-amino acids obtained from the procedure (A) and (B) were subjected to two-dimensional thin-layer chromatography on polyamide sheets (7.5×7.5 cm).⁴⁾ A solvent system of acetone-benzene-acetic acid (4: 2: 1, v/v) was used for the first dimension, and that of water-pyridine-acetic acid (10: 8: 1, v/v) for the second run at the right angle. The chromatogram of the FTH-amino acids were observed under long wave UV lamp.

The procedure (A) and (B) gave the same sequential informations. The total time for one cycle of reactions to yield the FTH-amino acid was about 3 hr. Some examples of chromatograms are illustrated in Fig. 1. FTH-Gln and -Glu have been clearly differentiated from each other. FTH-Leu and -Ile were not distinguished. Met and Tyr located at the C-terminal part were not detectable probably due to the loss at the step to remove FITC. Thus, the sequence of ten amino acids from

the N-terminus has been established as shown below.

H-His-Trp-Leu(Ile)-Gln-Leu(Ile)-Lys-Pro-Gly-Gln-Pro---

Accordingly, the structure of α substance-I_A should be expressed as follows.

H-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr-OH

The details of the experiments will be published later.

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