Isolation of a Novel Sex Hormone, Tremerogen A-10, Controlling Conjugation Tube Formation in *Tremella mesenterica* Fries

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Tremella mesenterica Fr., a Basidiomycete, is dimorphic, producing a homokaryotic yeast-like form as well as a typical dikaryotic mycelium and the basidiospores on dikaryotic mycelium germinate to form homokaryotic cells, which ordinarily propagate by budding.¹⁾ Between two compatible homokaryotic cells, the mating of both cells readily occurs leading to a dikaryotic mycelium. In the mating process of *T. mesenterica*, formation of conjugation tube is controlled by a single pair of alleles, *A* and *a* types, but production of a typical dikaryon is regulated by a series of "B factors".²⁾

In 1965, Bandoni found that the *A* and *a* mating type cells each produce the diffusing hormone(s) (erogen) effective in inducing the conjugation tube in the opposite type cell.³⁾ Recently Reid partially purified the hormone(s), which were suggested to be heterogeneous.⁴⁾

With a view to clarifying the mating process of *T. mesenterica* from a chemical stand point, we attempted to isolate the hormone produced by *A* type cell (IFO 559-1 9310) which induces the formation of conjugation tube in *a* type cell (IFO 559-8 9313). In this paper, we wish to report the isolation and some chemical properties of the hormone termed tremerogen A-10.

A type cells were cultured in a fermentation tank (200 liters) with aeration and agitation at 25°C for 42 hr, culture medium; 2.5% glucose, 0.5% peptone, 0.4% yeast extract, 0.1%

FIG. 1. Isolation Procedure of Tremerogen A-10.

KH_2PO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, pH 5.3. After filtration of the grown cells, the filtrate was concentrated *in vacuo* and then mixed with one tenth volume of *n*-butanol. The mixture was subjected to the isolation of tremerogen A-10 as illustrated in Fig. 1. The biological activity at each purification step was assayed by the method similar to that reported by Reid.⁴⁾

Tremmerogen A-10 was peculiarly adsorbed on some chromatographic materials. When adsorbed on Amberlite XAD-7 column, for example, the active principle could not be eluted with aqueous ethanol and, however, was completely recovered from the eluent with 40% aqueous ethanol containing 5% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. On Fractogel PGM column, the elution profiles were markedly effected with the composition of elution systems; water, methanol and acetic acid.

FIG. 2. UV Spectra of Tremmerogen A-10 and N-Acetyl-L-tyrosine Ethyl Ester.

—, tremmerogen A-10 in 80% methanol: acetic acid (99: 1) at a concentration of 1.0 mg/ml.
 -----, N-acetyl-L-tyrosine ethyl ester in the same solvent at a concentration of 93 µg/ml.

At the final step of purification, the chromatogram monitored by UV absorption at 280 nm showed a single peak which completely coincided with a peak of biological activity. Further, the isolated sample gave a single spot on each TLC using Silicagel (Merk) and Avicel (Funakoshi) plate with four kinds of solvent systems: 1) *n*-butanol: acetic acid: water (12: 3: 5), *R_f* 0.36 (Silicagel), 0.57 (Avicel); 2) *n*-butanol: *n*-propanol: 0.2 M acetic acid (2: 1: 3), *R_f* 0.05 (Silicagel), 0.46 (Avicel); 3) *sec*-butanol saturated with water *R_f* 0.16 (Silicagel), 0.55 (Avicel); 4) *n*-butanol: pyridine: acetic acid: water (15: 10: 3: 12) *R_f* 0.69 (Avicel). In our experiment, tremmerogen A-10 was a sole active substance in contrast to the result reported by Reid.⁴⁾ The isolated hormone, tremmerogen A-10, can induce the conjugation tube in *a* type cell at a concentration of 1~2 ng/ml.

Next we investigated the effects of various chemical reagents and enzymes on tremmerogen A-10 in order to get the clue about its chemical properties. When the hormone was incubated with a microbial protease, pronase (Kaken Kagaku), the biological activity became undetectable, suggesting that the substance is a peptide. Then tremmerogen A-10 was hydro-

lyzed with 5.7 N hydrochloric acid at 110°C for 20 hr and the hydrolyzate was applied onto amino acid analyzer (Hitachi K1a-3B). The molar ratios of detected amino acids were as follows: Asp 2.03, Ser 1.05, Glu 1.03, Pro 1.82, Gly 2.00, Ala 1.07, Tyr 0.93, His 1.05, Cys 0.74 (estimated from (Cys)₂ 0.37). As shown in Fig. 2, the UV spectrum of tremmerogen A-10 indicated that tryptophanyl residue was not contained in the molecule.

The biological activity of tremmerogen A-10 was totally retained after treatment with *N*-ethylmaleimide, indicating that sulfhydryl function is not involved. This fact was also confirmed by the quantitative analysis of sulfhydryl group using 5, 5'-dithio-bis (2-nitrobenzoic acid). Tremmerogen A-10 became inactive after treatment with performic acid whereas the absence of disulfide bond was implied by treatment of mercaptoethanol which had no effect on the biological activity of the hormone. Dansylation of the hormone followed by acid hydrolysis gave *N*-dansyl glutamic acid and suggested that *N*-terminous of the hormone is Glx. The biological activity of tremmerogen A-10 survived after treatment carboxypeptidase A (Miles Lab.), implying that C-terminus might be blocked.

Thus tremmerogen A-10 may be a dodecapeptide whose *N*-terminus is Glx and C-terminus is blocked. The sulfhydryl group in cysteinyl residue is possibly protected in the manner other than disulfide bond formation. The chemical structure of tremmerogen A-10 is now under investigation.

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REFERENCES

- 1) R. M. Whelden, *Mycologia*, **26**, 415 (1934).
- 2) R. J. Bandoni, *Can. J. Bot.*, **41**, 467 (1963).
- 3) R. J. Bandoni, *ibid.*, **43**, 627 (1965).
- 4) I. D. Reid, *ibid.*, **52**, 521 (1974).