**Induction of mitochondrial swelling by chaetochromin A, a
bis (naphtho- γ -pyrone) mycotoxin from *Chaetomium gracile***

II. Relationship with uncoupling effect

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gracile* 産生 bis (naphtho- γ -pyrone) マイコトキシン chaetochromin A
によるミトコンドリアの膨潤化誘起作用 II. 除共役作用との関係

Summary

Chaetochromin A has been studied in detail with regard to the swelling-inducing effect on mitochondria *in vitro* to gain insight into the interrelationship between its respiration-impairing and swelling-inducing activities.

Chaetochromin A elicited very rapid swelling of mitochondria in isotonic CsCl, RbCl, KCl, NaCl, and LiCl solutions, showing eminent differences in the magnitude and rate of swelling. The swelling amplitude was prominently larger in the KCl medium than those in the other kind of alkali metal chloride media, showing a distinct cation selectivity in the order of $K^+ \gg Cs^+$, Na^+ , $Rb^+ \gg Li^+$, and a sharp predominant K^+ -selectivity was observed in the initial rate of swelling.

Chaetochromin A was tested for the induction of swelling in the isotonic KCl medium at various pH between pH 5 and 9. The amplitude and velocity of swelling were markedly increased corresponding to the decrease of the pH in the test system. A pH optimum for the swelling induction seemed to locate at much lower pH than the physiological pH range.

Chaetochromin A, a bis (naphtho- γ -pyrone) mycotoxin from *Chaetomium gracile*¹⁾, has been shown to elicit marked swelling of mitochondria in liver and heart muscle *in vivo*²⁾ and *in vitro*³⁾, showing a good correspondence between the results of both *in vivo* and *in vitro* experiments. It has been shown to impair the respiratory system of rat liver mitochondria *in vitro* causing the uncoupling of oxidative phosphorylation and marked depression of both succinate- and NAD-linked respirations at the similar level of concentrations to those for the swelling induction⁴⁾. The knowledge on the interrelationship between the uncoupling effect and the swelling-inducing activity of chaetochromin A is however not available. It is therefore of much interest to know whether the swelling (loss of the struc-

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tural integrity) precedes the uncoupling of respiration (loss of the functional integrity) in mitochondria or *vice versa*.

In the present study, the effects of alkali metal ions and pH on the chaetochromin A-induced swelling were examined using isolated rat liver mitochondria and the correlation between the uncoupling effect and the swelling-inducing activity of chaetochromin A was discussed.

Materials and Methods

Reagents Chaetochromin A was isolated from *C. gracile*¹⁾ and dissolved in freshly redistilled N,N-dimethylformamide. Flavoglaucin (from *Eurotium chevalieri*) was a gift of Dr. J. Kitamura of Gifu University of Pharmacy and used as a solution of N,N-dimethylformamide. Tris-(hydroxymethyl)aminomethane was purchased from Sigma Chemical Co. Bovine serum albumin (BSA, fraction V) was the product of Armour Pharmaceutical Co. All other reagents were of the purest grade commercially available.

Procedures Rat liver mitochondria were prepared according to the method of Schneider⁵⁾ using a preparation medium composed of 0.25 M sucrose, 0.5 mM EDTA, and 10 mM Tris-HCl (pH 7.4). All procedures were performed at 0–4°C. Mitochondrial respiration was tested using oxygen electrode system⁴⁾ and mitochondria showing tightly coupled respiration were employed in the experiments.

Mitochondrial swelling was monitored by measuring the absorbance decrease of mitochondrial suspension using a recording spectrophotometer UVIDEC 610C (JASCO) according to the finding by Tedesch and Harris⁶⁾ in the correlation between mitochondrial swelling and absorbance decrease. In the present study, the absorbance decrease was followed at 700 nm instead of 520 nm to minimize the influence of light absorption by chaetochromin A, whose maximal absorption wavelength was located at 412 nm, added to the system. The higher concentrations of chaetochromin A were therefore requested to observe the swelling-induction at the initial absorbance at around 0.8. The incubation was carried out at room temperature (28°C) in a cuvette equipped in the recording spectrophotometer.

Mitochondrial protein was assayed by the method of Lowry et al.⁷⁾ by means of BSA as a standard protein.

Results

Chaetochromin A-induced swelling in various alkali metal chloride media

Mitochondrial swelling was induced by chaetochromin A in the 0.15 M LiCl, NaCl, KCl, RbCl, and CsCl media, which contained 20 mM Tris-HCl (pH 7.4), and the initial rate and magnitude of swelling were compared (Fig. 1). The addition of chaetochromin A to mitochondrial suspension caused the marked and very quick decrease of absorbance, indicating the induction of very swift swelling of mitochondria and the quick swelling was taken place by a very slow-speed swelling very soon. As seen in the Fig. 1, apparent differences were observed in the magnitude of swelling; the largest in the KCl medium and the smallest in the LiCl medium. No significant difference was seen in the NaCl, RbCl, and

Fig. 1. Chaetochromin A-induced swelling of mitochondria in a series of alkali metal chloride solutions.

Reaction medium was composed of 0.15 M LiCl, NaCl, KCl, RbCl, or CsCl and 20 mM Tris-HCl (pH 7.4) in a final volume of 2.0 ml. Mitochondrial protein was about 0.52 mg/ml giving initial absorbance of 0.85.

Fig. 2. The relationship between the initial swelling rate and the reciprocal values of ionic radius of each alkali metal ion.

Reaction condition was the same as in Fig. 1 except the chart-speed (50 mm/min). The concentration of flavoglaucin was 60 μ M. The initial swelling rate was calculated from delta-absorbance for two min after the addition of chaetochromin A or flavoglaucin.

CsCl media.

The initial rates of chaetochromin A-induced swelling were compared with those induced by flavoglaucin, a benzohydroquinone mycotoxin which is a potent inducer of mitochondrial swelling and is an uncoupler of oxidative phosphorylation⁹, in the isotonic alkali metal chloride media (Fig. 2).

The initial swelling rate was measured by following the absorbance decrease at higher chart speed (50 mm/min) of the recording spectrophotometer (data not shown) and the relationship between the initial swelling rate and the reciprocal value of ionic radius of each

Fig. 3. Chaetochromin A-induced swelling of mitochondria at various pH.

Swelling was induced in 0.15 M KCl medium changing the pH between 5 and 9. Numbers at the end of each curve show the pH of the medium.

alkali metal ion were depicted in Fig. 2. The swelling rate in KCl medium was much larger than those of other alkali metal chloride solutions. Such strong K^+ -selectivity for the swelling induction was not observed for flavoglauconin.

Chaetochromin A-induced swelling at various pH

It is now well documented that uncouplers, which act as proton-conductors across mitochondrial inner membrane, exhibit maximal activity at around their pK_a values; usually physiological pH range. Chaetochromin A was therefore examined for the swelling-inducing activity in the isotonic KCl medium at various pH between pH 5 and 9 to find its optimal pH (Fig. 3). Freshly prepared mitochondria did not show the absorbance decrease (swelling) during the incubation without chaetochromin A at the pH range tested.

The magnitude of chaetochromin A-induced swelling was conspicuously amplified corresponding to the decrease of pH in the test system, suggesting that the non-charged form of chaetochromin A may be more favorable than the charged (anionic) form for the induction of mitochondrial swelling. In this connection, the pK_a value of chaetochromin A is located at about pH 7.5.

Discussion

The mode of action of chaetochromin A on isolated rat liver mitochondria has been investigated in detail with regard to the swelling induction to gain insight into the molecular mechanism for the toxic action to mitochondrion and also to know the interrelationship between the uncoupling effect on oxidative phosphorylation and the swelling-inducing activity of chaetochromin A.

It has been established by the present study that chaetochromin A induces a drastic swelling of mitochondria in isotonic alkali metal chloride solutions, showing a distinct cation

selectivity sequences of $K^+ \gg Na^+$, Rb^+ , $Cs^+ \gg Li^+$ in the swelling magnitude and of $K^+ \gg Cs^+$, Li^+ , Rb^+ in the initial swelling rate. This result may suggest that chaetochromin A binds to membrane protein(s) or phospholipids and forms a K^+ -selective carrier complex or channel. This presumption may be strongly supported by the evidence that chaetochromin A-induced swelling is completely inhibited by magnesium ion⁸⁾ as reported for the valinomycin-induced swelling⁹⁾.

Chaetochromin A-induced swelling showed marked pH-dependent alterations in the magnitude, extending the swelling magnitude corresponding to the decrease of pH of the system, and the maximal swelling was seen at below pH 6.0 which was critically lower than the pKa value of chaetochromin A. It was thus speculated from this observation that non-charged form of chaetochromin A exhibited stronger activity than its charged (anionic) form in the swelling induction against the concept for its uncoupling effect. It is now widely understood that the molecular mechanism of uncoupling is discharge of a membrane potential existing across a proton-impermeable membrane and the discharge is brought up by uncouplers which act as non-vectorial proton conductors across the energy-conserving membrane¹⁰⁻¹³⁾. Uncouplers exhibit maximal uncoupling effect at around pKa values¹⁴⁻¹⁷⁾ in which equimolar amount of anionic and non-charged forms of uncouplers are present in the reaction system. On this point of view, chaetochromin A may exert the maximal uncoupling effect at around pH 7.5.

In addition, the impairing effect of chaetochromin A on mitochondrial respiration was observed in the reaction medium containing 5 mM $MgCl_2$ ⁴⁾. Whereas the swelling-induction was completely inhibited by $MgCl_2$ ⁸⁾. From these results and considerations, it was concluded that the molecular mechanisms for the uncoupling effect and swelling-inducing effect of chaetochromin A were not identical and the uncoupling of oxidative phosphorylation was not the priming reaction for the initiation of mitochondrial swelling.

References

- 1) Koyama, K., Natori, S.: Chem. Pharm. Bull., **35**, 578 (1978).
- 2) Ohtsubo, K.: J. Toxicol. Pathol., **2**, 163 (1989).
- 3) Kawai, K., Mori, S., Hisada, K., Nozawa, Y., Koyama, K., Natori, S.: Proc. Jpn. Assoc. Mycotoxicol., **33**, 25 (1991).
- 4) Kawai, K., Hisada, K., Mori, S., Nozawa, Y., Koyama, K., Natori, S.: Proc. Jpn. Assoc. Mycotoxicol., **33**, 31 (1991).
- 5) Schneider, W. C.: J. Biol. Chem., **176**, 259 (1948).
- 6) Tedesch, H., Harris, D. L.: Arch. Biochem. Biophys., **58**, 52 (1955).
- 7) Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J.: J. Biol. Chem., **193**, 265 (1951).
- 8) Kawai, K., Mori, H., Kitamura, J.: Toxicol. Lett., **19**, 321 (1983).
- 9) Ligeti, E., Fomyo, A.: FEBS Lett., **79**, 33 (1977).
- 10) Lehninger, A. L.: Physiol. Rev., **42**, 467 (1962).
- 11) Mitchell, P.: Biol. Rev., **41**, 445 (1966).
- 12) McLaughlin, S. G. A., Dilger, J. R.: Physiol. Rev., **60**, 825 (1980).
- 13) Terada, H.: Biochim. Biophys. Acta, **630**, 225 (1980).
- 14) Hopfer, U., Lehninger, A. L., Thompson, T. E.: Proc. Natl. Acad. Sci. USA, **59**, 484 (1968).
- 15) LeBlanc Jr, O. H.: J. Membr. Biol., **4**, 227 (1971).
- 16) Finkelstein, A.: Biochim. Biophys. Acta, **205**, 1 (1970).
- 17) Skulachev, V. P., Sharaf, A. A., Liberman, E. A.: Nature, **216**, 718 (1967).