SWELLING AND CONTRACTION OF CUCUMBER HYPOCOTYL MITOCHONDRIA
YEONG-JING CHENG* KUAN-JEN YANG**

INTRODUCTION

Mitochondria, the power-house in a cell, are present in the cytoplasm of all eukaryotic cells. It located near the structure that require ATP or structure as a source of fuel, on which all cellular metabolism and biosynthesis can be performed. Owing to their important functions, they undergo rhythmic energy-dependent volume changes. These volume changes depend on respiration and electron transport and are easily measured optically as changes in light scattering.

Most studies on mitochondria indicate that, in terms of fundamental structure and respiratory properties, plant and animal mitochondria are alike; however, recent studies show that there are many differences in swelling-contraction activity and respiratory properties between plant and animal mitochondria. For example, animal mitochondria do not swell or accumulate ions when they respire in a buffered medium of 0.1 M KCl (Brierley, 1970), while plant mitochondria swell spontaneously in buffered KCl (Earnshaw and Truelove, 1968; Stoner and Hanson, 1966; Wilson et al., 1969). In other words, animal mitochondria swell actively (substrate-dependent), whereas plant mitochondria passively.

In recent years, several studies on the swelling-contraction properties of plant mitochondria have been done, such as bean (Earnshaw and Truelove, 1968; Malnotra and Spencer, 1970), and corn (Kenevick and Hanson, 1966; Lorimer and Miller, 1969; Müller et al., 1970; Stoner and Hanson, 1966; Wilson et al., 1969). They reported that corn and bean mitochondria swell spontaneously in buffered KCl and contract rapidly not only with ATP, but also with the addition of an oxidizable substrate such as succinate. Some investigators (Stoner et al., 1964; Truelove and Hanson, 1966) also demonstrated that the substrate-induced contraction was prevented by inhibitors of electron transport, while ATP-powered contraction was not.

So far only the properties of mitochondria from corn and bean have been well characterized. This suggest that more studies on swelling and contraction phenomena in other plant materials are needed. In this paper, we shall show that cucumber hypocotyl mitochondria will contribute some different swelling-contraction phenomena compare with corn mitochondria.

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MATERIALS AND METHODS

Isolation of Mitochondria:

The cucumber seeds (Cucumis sativus L.) were soaked in tap water for 2 hr and then planted in vermiculite. The seedlings were grown in the dark at 26-28°C. The 4-day-old etiolated hypocotyls were harvested and mitochondria were isolated from 50g/m tissue according to the method of Malhotra and Spencer (1970). The hypocotyls were grinded with a mortar and pestle for 2 min in 100 ml of grinding medium (0.5M mannitol, 0.001M EDTA, 0.1% BSA, 0.05% cysteine, and 0.05M Tris-HCl buffer, pH 7.4). The homogenate was passed through four layers of cheesecloth and the filtrate was centrifuged at 2,500 g for 5 min. The pellet was discarded and the supernatant was then centrifuged again at 12,000 g for 10 min. The mitochondrial pellet was suspended in 0.4M sucrose. All steps during isolation were carried out at 0-4°C.

Measurement of Swelling and Contraction:

Swelling and contraction of mitochondria were determined by measuring the absorbance at 520 nm with a SHIMADZU QV-50 spectrophotometer fitted with a controlled temperature cell housing and U-100 recorder. Changes in volume were indicated by changes in light scattering at 520 nm (Bier, 1957). The detail procedure for measuring swelling and contraction was as add, at zero time, 0.1 ml of mitochondrial suspension (0.4-0.5 mg protein) to a cuvette containing 2.9 ml of 0.1 M KCl, 1 mg/ml BSA, 0.02M Tris-HCl buffer, pH 7.5(Earshaw and Truelove, 1968) with any other additions as indicated. The initial reading was taken after 1 min and each subsequent reading was taken at 5-min intervals. After mitochondrial swelling terminated, contraction was then initiated by the addition of a small volume (0.05-0.10 ml) of an oxidizable substrate, ATP+Mg++ or ADP. If succinate was used, the final concentration was 10 mM. The first reading after addition of succinate or ATP was taken after 1 min and subsequent reading were taken at 5-min intervals until contraction terminated. A decrease in absorbance indicate swelling, and an increase indicate contraction.

The mitochondrial protein was determined by the procedure of Lowry et al. (1951) with bovine serum albumin standard.

RESULTS AND DISCUSSION:

Substrate- and ATP-induced Contraction:

When cucumber hypocotyl mitochondria suspended in sucrose were transferred to a Tris-medium containing KCl and bovine serum albumin, it swell spontaneously. The swelling phase was usually complete in 20 minutes. At this moment, the addition of contracting agents, such as succinate, NADH, ADF and ATP, caused a sharp increase in absorbance at 520 nm. Fig 1 shows that oxidizable agents, NADH and succinate, produce more rapid and high level of contraction than ATP. According to our observation, the level of contraction of cucumber mito-
chondria is maintained as long as there is sufficient oxygen and substrate in the medium, otherwise reswelling will be occured. Similar results have been reported for mitochondria from maize and bean (Earnshaw and Truelove, 1968; Hanson et al., 1968).

**Effect of Varying KCl Concentration**

Figure 2 shows the responses when the mitochondria suspended in 0.4 M sucrose were injected into cuvette with KCl concentration varying from 0 to 600 mM. It is obvious that the extent of swelling is in proportion to the concentration of KCl and 400 mM of KCl gives a maximal swelling. Stoner and Hanson (1966) assumed that this spontaneous swelling is accompanied by penetration of the suspending solute and that there is a rapid osmotic adjustment which follows solute penetration.

The results in the experiments also indicate that concentration of KCl greater than 400 mM may lower the extent of swelling as a result of damage to the osmotic mechanism(s) of mitochondria.

**Effect of Varying pH**

Figure 4 shows levels of swelling at varying pH. When the pH of the medium changed from 6.0 to 9.0, the extent of swelling increased by approximately 100%. Minimal swelling occurred at acidic pH and maximal at alkaline pH (Fig 6). It is clear from the data that both acidic and alkaline pH retarded contraction (Fig 5).

These facts probably indicate that the swelling is a passive process, which relate to the pH of medium, and contraction is an active process, which link to either the ATP hydrolysis or substrate oxidation. Blackman and Moreland (1971) reported that ATPase activity increased with increasing pH and reached an optimum at pH 7.5. Previous study, by Ikum (1970) also indicated that pH below 7.2 retarded succinate and malate oxidation. All of these data suggested that the contraction is associated with the enzymatic reactions and is pH dependent.

**Effect of Ca++ and Inorganic Phosphate**

Isolated cucumber mitochondria swell in buffered KCl and KH$_2$PO$_4$ (1 mM without KCl) solutions, but not in Ca++ (without KCl) solution (Fig 7). In the buffered KCl, both Ca++ and phosphate promote swelling. Fig 8 and Fig 9 show that the promotion of swelling increase with increasing both Ca++ and phosphate concentration from 1 mM to 4 mM and reached the maximal level at 4 mM. The higher concentration of Ca++ and phosphate (10 mM), however, lower the promotion. 4 mM Ca++ plus phosphate gives no striking increase in promotion of swelling. In earlier study on corn mitochondria by Truelove and Hanson (1966) indicated that the phosphate has no important effect on swelling, but Ca++ retard the rate and extent of swelling. Obviously, our observations are different from their opinions. Perhaps, the difference may be due to the special characteristics of different plant materials, but the actual reasons still in doubt.

In the subsequent studies on contraction show that phosphate inhibits contraction and calcium alone slightly promotes it (Fig 10) as previous.
Swelling and Contraction of Cucumber Hypocotyl Mitochondria

Truelove and Hanson (1966) demonstrated that during the period when contraction occurs, phosphate and calcium are taken up and accumulated. Considerable evidences have been proposed to support the concept of energy-linked cations and anions transport. Hanson and Miller (1967) and other authors (Elzam and Hodges, 1968; Kenefick and Hanson, 1966; Lardy and Ferguson, 1969; Mela, 1969; Miller et al., 1970) described calcium and magnesium effects on swelling, contraction, phosphate-uptake and oxidative phosphorylation in maize mitochondria. From their results, they concluded that Ca\(^{++}\) activates phosphate transfer by forming an unstable complex with a phosphorylated high-energy intermediate causing it to break down and give rise to calcium and phosphate transport. The overall diagram proposed are describe below:

![Diagram of the metabolism process involving substrate, coupling site, oxygen, swelling, contraction, ATP, ADP, Pi, DNP, Mg\(^{++}\), Ca\(^{++}\), and X as intermediates.]

Table I: Effects of Pi and Ca\(^{++}\) on the swelling and contraction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Swelling</th>
<th>Contraction</th>
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<tbody>
<tr>
<td></td>
<td>A.O.D. %</td>
<td>A.O.D. %</td>
</tr>
<tr>
<td>Control</td>
<td>0.069</td>
<td>0.066</td>
</tr>
<tr>
<td>1 mM Ca(^{++}) NO3</td>
<td>0.038</td>
<td>0.056</td>
</tr>
<tr>
<td>1 mM Pi</td>
<td>0.081</td>
<td>0.056</td>
</tr>
<tr>
<td>1 mM Pi</td>
<td>0.080</td>
<td>0.056</td>
</tr>
<tr>
<td>2 mM Pi</td>
<td>0.108</td>
<td>0.044</td>
</tr>
<tr>
<td>10 mM Pi</td>
<td>0.100</td>
<td>0.028</td>
</tr>
<tr>
<td>1 mM Ca(^{++})</td>
<td>0.087</td>
<td>0.068</td>
</tr>
<tr>
<td>10 mM Ca(^{++})</td>
<td>0.010</td>
<td>0.073</td>
</tr>
<tr>
<td>10 mM Pi + Ca(^{++})</td>
<td>0.092</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Effect of Growth Regulators and Inhibitors

The effects of animal hormones on the swelling activity of mitochondria from various animal tissues have been well established and are permitted in several reviews (Lehninger, 1964; Lindberg et al., 1961).
Lehninger (1961) proposed that the mitochondria swelling induced by thyroxine and other hormones may be associated with the physiological actions of these hormones. The physiological consequence of thyroxine-induced swelling probably involve changes in permeability and transport phenomena. So as do other animal hormones. In recent studies on the swelling-contraction of plant mitochondria, however, relatively little work has been done concerning the action of plant growth regulators, such as auxins, gibberellins and cytokinins. In our preliminary studies show that there is some promotion or inhibition has been found after the administration of various concentration of IAA and GA. However, high concentration of 6-benzylaminopurine and 2,4-D slightly promote swelling but have no any effect on contraction. When BA is used, the promotion of swelling activity initially slow, then strikingly after 10 minutes (Fig 11). While 2,4-D gives a slightly promotion through the process.

Chelating agents, such as citrate and EDTA, give a pronounced inhibition on swelling and a striking promotion on contraction of cucumber mitochondria (Fig 12 and 13). Its inhibition on swelling and promotion on contraction probably due to the removal and activation of some factors which necessary for the process of swelling and contraction.

Cyanide, an inhibitor of electron transport, strongly inhibits swelling and succinate-powered contraction by blocks the electron transfer and respiration (Fig 14). However, cyanide has little effect on ATP-powered contraction (Fig 15) as described by several investigators (Keneick and Hanson, 1966; Miller and Koepppe, 1971; Stoner and Hanson, 1966). Other respiratory inhibitors such as antimycin A, not only blocks electron transfer but also inhibits contraction of mitochondria (*Wilson and Bonner, Jr., 1970*).

DNP, an uncoupling agent of oxidative phosphorylation, has been reported to stimulate ATPase activity (*Blackman and Moreland, 1971*). With low concentration of DNP (100 uM), it accelerates swelling but higher concentration of DNP (10 mM) inhibits both swelling and contraction. This experiment gives a excellent agreement with previous studies by Hanson et al. (1965; 1968). The effects of some respiratory inhibitors and uncoupling agent on swelling and contraction of cucumber hypocotyl mitochondria are summarized in Table II.

### Table II: Effects of inhibitors on the swelling and contraction.

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Swelling $I_{50}$</th>
<th>Contraction $I_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.086</td>
<td>0.0070</td>
</tr>
<tr>
<td>100 uM EDTA</td>
<td>0.039</td>
<td>0.0110</td>
</tr>
<tr>
<td>1 mM Citrate</td>
<td>0.026</td>
<td>0.0129</td>
</tr>
<tr>
<td>10 mM KCl</td>
<td>0.058</td>
<td>0.129 184</td>
</tr>
<tr>
<td>10 mM EDTA</td>
<td>0.044</td>
<td>0.128 183</td>
</tr>
<tr>
<td>10 mM KCl</td>
<td>0.020</td>
<td>0.124 180</td>
</tr>
<tr>
<td>10 mM KCl</td>
<td>0.048</td>
<td>0.004 6</td>
</tr>
<tr>
<td>10 mM DNP</td>
<td>0.045</td>
<td>0.045 64</td>
</tr>
<tr>
<td>10 mM Antimycin</td>
<td>0.050</td>
<td>0.70</td>
</tr>
</tbody>
</table>

**Abbreviations:**
- EDTA: Ethylenediamine Tetraacetic Acid, Disodium Salt
- BSA: Bovine Serum Albumin
- Tris: Tris (hydroxymethyl) aminomethane
- DNP: 2,4-Dinitrophenol
- IAA: Indole-3-Acetic Acid
- GA: Gibberellic Acid
- BA: 6-Benzylaminopurine
- 2,4-D: 2,4-Dichlorophenoxyacetic Acid
LITERATURE CITED


Fig. 1. Contraction of cucumber mitochondria by various contracting agents after swelling in the basic medium of 0.1 M KCl, 0.02 M Tris-HCl, 1 mg/ml BSA, pH 7.5.

Fig. 2. Effect of varying KCl concentration on the swelling of cucumber mitochondria. The medium was 0.02 M Tris-HCl, 1 mg/ml BSA, pH 7.5 with KCl as indicated.

Fig. 3. Effect of KCl concentration on swelling of cucumber mitochondria.

Fig. 4. Effect of pH on swelling of mitochondria. The mitochondria were suspended in the medium the same as Fig. 1 with varying pH.
Fig. 5. Effect of pH on the contraction of mitochondria. Contraction was initiated after swelling in the basic medium the same as Fig. 1 with varying pH, by the addition of 0.1 ml succinate, gives a final concentration of 10 mM.

Fig. 6. Swelling and contraction as a function of pH. The curves present changes in O.D. 20 minutes after the initiation of swelling and contraction.

Fig. 7. Isolated cucumber mitochondria swell in buffered 0.1 M KCl & 1 mM K phosphate (without KCl) solution, but not in Ca²⁺ (without KCl) solution.

Fig. 8. Effect of varying Pi concentration on the swelling. The medium was the same as Fig. 1 with varying concentration of Pi as indicated.
Fig. 9. Effect of varying Ca++ concentration on the swelling. The medium was the same as Fig. 1 with various Ca++ concentration as indicated.

Fig. 10. Effect of calcium and phosphate on the contraction. Contraction was initiated after swelling 20 minutes in the medium the same as Fig. 1 with calcium and phosphate as indicated, by the addition of 0.1 ml succinate (0.3M), pH 7.3.

Fig. 11. Effect of BA and 2,4-D on the swelling of cucumber mitochondria. The mitochondria were injected at zero time into the cuvette containing 0.02 M Tris-HCl, 1 mg/ml BSA, 0.1 M KCl, pH 7.3.

Fig. 12. Effect of chelating agents on the swelling of cucumber mitochondria. The medium was the same as Fig. 1 with citrate and EDTA as indicated.
Fig. 13. Effect of chelating agents on the contraction of cucumber mitochondria. Contraction was initiated after swelling 20 minutes in the medium the same as Fig. 1 with chelating agents as indicated, by 0.1 ml 0.3 M succinate.

Fig. 14. The inhibition of cyanide on swelling. Swelling was initiated by the addition of 0.1 ml mitochondrial suspension, and the medium was the same as Fig. 1 with KCN as indicated.

Fig. 15. The inhibition of cyanide and antimycin A on contraction. Contraction was initiated after 20 minutes of swelling by the addition of 0.1 ml 0.3 M succinate or 0.09 M ATP.
中文摘要

小黃瓜粒線體脹縮性之研究

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小黃瓜粒線體以等滲液（0.4 M蔗糖溶液）自細胞分離後，若有可被氧化受質如NADH, Succinate和ATP存在時，可進行急速收縮。若粒線體置於氯化鉀緩衝液中時，即迅速膨脹。此膨脹性受氧化鉀液濃度及pH影響。鈣離子和無機磷酸離子亦能促進膨脹；此外植物生長調節剤如6-Benzylaminopurine和2,4-D在適當濃度時亦有促進膨脹之功能。呼吸抑制剤Cyanide和Animycin A不但抑制粒線體的膨脹，而且抑制其收縮，故粒線體之膨脹與細胞呼吸及氧化磷酸化作用有密切關係。