

## **A study on the activities of carbonic anhydrase of two species of bryophytes, *Tortula sinensis* (Müll. Hal.) Broth. and *Barbula convoluta* Hedw.**

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(Received 23 January 2005, accepted 4 September 2005)

**Abstract** – We report our studies of the activities of carbonic anhydrase (CA) in two limestone mosses, *Tortula sinensis* (Müll. Hal.) Broth. and *Barbula convoluta* Hedw., and the influence of desiccation and salinity on their CA activities. The result shows that the activities of carbonic anhydrase can be measured in these two mosses, and the activity of extracellular carbonic anhydrase in *T. sinensis* and in *B. convoluta* was 37.96% and 41.25% of the total CA activity, respectively. The activity of carbonic anhydrase in *B. convoluta* and *T. sinensis* reaches its peak when the concentration of sodium chloride is 2% (w/v) and 1% (w/v), respectively. The relationship between the dehydration time and the activities of carbonic anhydrase in mosses is one of parabola. The carbonic anhydrase activity in *T. sinensis* reaches its maximum after 2 hours of dehydration, and after 3 hours, in the case of *B. convoluta*. The study of the activities of carbonic anhydrase in *T. sinensis* and *B. convoluta* may explain how these two mosses adapt to growing on limestone.

**Bryophyte / moss / carbonic anhydrase / limestone / *Tortula sinensis* / *Barbula convoluta***

### **INTRODUCTION**

Carbonic anhydrase (CA; EC 4.2.1.1) is a zinc-containing metalloenzyme that catalyzes the reversible conversion of CO<sub>2</sub> to bicarbonate. It is widely distributed in animals, plants, archea and eubacteria, where it is involved in diverse physiological processes, such as ion exchange, acid-base balance, carboxylation/decarboxylation reactions and inorganic carbon diffusion between the cell and its environment as well as within the cell (Badger & Price, 1994; Sasaki *et al.*, 1998; Kaplan & Reinhold, 1999).

In some aquatic bryophytes such as *Riccia fluitans*, *Fontinalis antipyretica* and some hornworts, carbonic anhydrase was reported to play a role in the CO<sub>2</sub>-

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concentrating mechanism in these plants (Bain & Proctor, 1980; Ballesteros *et al.*, 1998; Hanson *et al.*, 2002). In terrestrial plants, the variations of CA activity in leaves may result in the changes of spatial distribution of micro-environmental concentration of CO<sub>2</sub> (Gillon & Yaki, 2001). Moreover, CA may facilitate the dissolution of limestone (Liu, 2000). These facts suggest that root-induced acidification of the rhizosphere is not the only factor causing the dissolution of calcium carbonates (Jaillard *et al.*, 1991), and CA in terrestrial mosses may be another cause for the dissolution of calcium carbonates.

As far as we know, no study on CA activity of terrestrial mosses, especially those growing on rock, has been reported in the literature. *Tortula sinensis* and *Barbula convoluta* are two mosses often found on limestone or dolomite beds (Zhang, 1997). This paper reports on how the activity of CA in *T. sinensis* and *B. convoluta* can be assayed, and discusses the relationship between the activities of CA in those mosses and their habitats.

## MATERIAL AND METHODS

*Tortula sinensis* and *B. convoluta* were collected in May of 2004 from the limestone bed of Lover Valley in Guiyang, Southwest China, for the study. These mosses were dealt with as follows:

*Treatment 1:* *T. sinensis* and *B. convoluta* were placed separately in Petri-dishes containing an aqueous solution of an inhibitor of extracellular CA—acetazolamide (AZ) (30 mM). The samples were then incubated for 1 h with illumination intensity and temperature set at  $34 \mu\text{molm}^{-2}\text{s}^{-1}$  and 25 °C, respectively. The control samples of *T. sinensis* and *B. convoluta* were treated in the same way except that AZ was omitted and distilled water was used.

*Treatment 2:* Plants of *T. sinensis* and *B. convoluta* placed in dry Petri-dishes were air-dry and allowed to dehydrate for varying periods of 0, 0.5, 1, 2, 3, 4, 5 h, with the illumination intensity and temperature set at  $34 \mu\text{molm}^{-2}\text{s}^{-1}$  and 25 °C, respectively.

*Treatment 3:* Plants of *T. sinensis* and *B. convoluta* were incubated for 1 h in Petri-dishes containing different concentrations of sodium chloride solutions (0%, 0.5%, 1%, 2%, 5%, and 10%) (w/v) with the illumination intensity and temperature set at  $34 \mu\text{molm}^{-2}\text{s}^{-1}$  and 25 °C, respectively.

The activities of total and extracellular CA of intact plants of *T. sinensis* and *B. convoluta* were assayed thereafter. Each of the differently treated moss samples (1.0 g) was quickly frozen in liquid N<sub>2</sub>, ground into powder with a mill, and then homogenized with 3 mL of extraction buffer (10 mM veronal buffer with 50 mM 2-mercaptoethanol, pH 8.2). The homogenate was centrifuged at 10,000 g for 5 min. The supernatants from AZ-free treatments (incubated in distilled water) were used for measurement of total CA activity. Acetazolamide (AZ), an 'impermeant-inhibitor' of CA, is suitable for identifying extracellular CA, if used at low concentration for short times (Ghoshal *et al.*, 2002). Therefore, the extracellular CA activity was determined as the difference in CA activities between the supernatants of the AZ-free and AZ-treated moss samples.

The CA activity was electrochemically determined as the time required for the pH of the enzyme-buffer mixture to decrease from 8.2 to 7.2 in 15 mL ice-cold barbital buffer (20 mM, pH 8.30) with the above 1.0 mL supernatants, and with 10 mL of ice-cold CO<sub>2</sub>-saturated distilled H<sub>2</sub>O added into it (Wilbur and

Anderson, 1948). One unit (WA-unit) of activity was defined as:  $WA = t_0/t - 1$ , where  $t_0$  and  $t$  are the times required for the pH changes, respectively, in the enzyme-free buffer (control) and buffer mixture containing enzyme sample.

The rate of water loss was determined as plant weight loss after different dehydration times and defined as:  $W_1 = W_i/W_0 \times 100\%$ , where  $W_1$  is the rate of water loss,  $W_i$  is the moss weight when time equals to  $i$ , and  $W_0$  is the original weight of the moss.

## RESULTS

### The activities of CA of *T. sinensis* and *B. convoluta*

Table 1 shows the activities of CA of *T. sinensis* and *B. convoluta*. It can be seen from Table 1 that the activity of CA of *B. convoluta* was greater than that of *T. sinensis*, and that the presence of AZ lowers CA activity in both mosses. Our experiments showed further that 37.96% of the total CA activity in *T. sinensis* and 41.25% in *B. convoluta* could be attributed to the activity of the extracellular CA.

### The influence of the duration of dehydration on the CA activity of *T. sinensis* and *B. convoluta*

The rate of water loss in both moss species studied varied with the length of dehydration time (Fig. 1). During the first h, the rates of dehydration of both *T. sinensis* and *B. convoluta* were rapid; but slowed down thereafter. The rate of water loss of *B. convoluta* was greater than that of *T. sinensis*.

Fig. 2 shows the influence of the duration of dehydration on the activity of CA in both *T. sinensis* and *B. convoluta*. There was a parabolic relationship

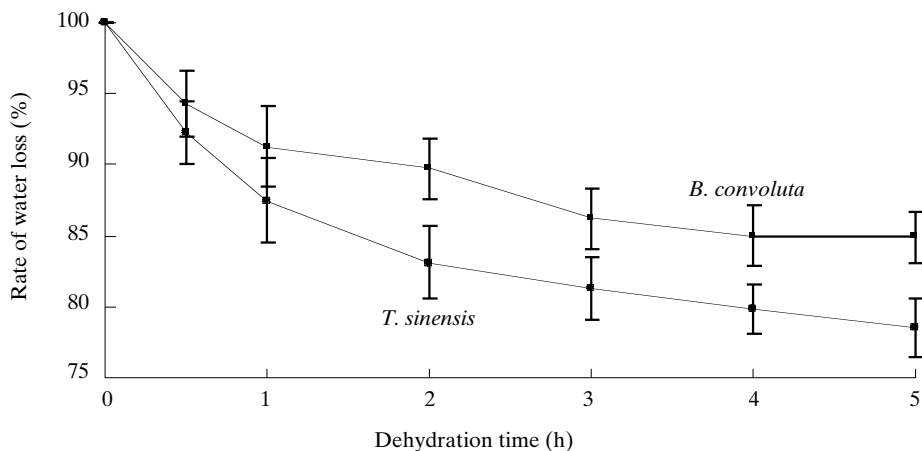


Fig. 1. The rate of water loss ( $W_1$ ) ( $M \pm SD$ ,  $n = 5$ ) of *T. sinensis* and *B. convoluta* at different dehydration times.

between the duration of dehydration and the activities of CA in the two species of mosses studied. The CA activity of *T. sinensis* reached its maximum in 2 h, whereas the CA activity of *B. convoluta* reached its maximum after 3 h of dehydration.

### The influence of salinity stress on the CA activity of *T. sinensis* and *B. convoluta*

Fig. 3 shows the activity of CA of *T. sinensis* and *B. convoluta* at different concentrations of sodium chloride. In the case of *B. convoluta*, the activity of CA peaked when the moss was exposed to 2% (w/v) sodium chloride, and dropped at higher concentrations of sodium chloride. The same changes for CA activity were observed in *T. sinensis*; the only difference was that the activity of CA of *T. sinensis* peaked in 1% (w/v) sodium chloride.

## DISCUSSION

Many mosses can live on limestone, mainly because they can adapt themselves to water stress (Stark, 1983; Eldridge & Tozer, 1997). The mechanism of this adaptation involves increased water absorption and reduced transpiration (Richardson, 1981; Zhang *et al.*, 2002). However this does not explain why some mosses can tolerate long-term dehydration. It seems that some other clues can be found in this study.

*Riccia fluitans* L., one of aquatic bryophytes, was reported to have no extracellular CA activity (Ballesteros *et al.*, 1998). On the other hand, extracellular CA activity was reported to be less than 15% of total cellular activity in some higher plants such as wheat, spinach, *Moricandia arvensis*, and *Mesembryanthemum crystallinum* (Tsuzuki *et al.*, 1985).

Both *T. sinensis* and *B. convoluta* have active carbonic anhydrase and a high proportion of extracellular carbonic anhydrase activity (see Table 1). The activity of extracellular CA in *T. sinensis* and in *B. convoluta* was found to be 37.96 % and 41.25% of the total CA activity, respectively (see Table 1). Extracellular CA is a major component of the CO<sub>2</sub>-concentrating mechanism in some algal species (Aizawa & Miyachi, 1986). Therefore, higher activity of extracellular CA facilitates the production of soluble bicarbonate which can be transformed rapidly into H<sub>2</sub>O and CO<sub>2</sub>; these can then be transported to mesophyll cells in the leaf to compensate for the shortage of water and CO<sub>2</sub> that are needed for photosynthesis under water stress.

Table 1. The activity of carbonic anhydrase of *T. sinensis*, *T. sinensis* +AZ, *B. convoluta* and *B. convoluta* +AZ

	Total CA activity WAU.gFW <sup>-1</sup>	+AZ CA activity WAU.gFW <sup>-1</sup>	Extracellular CA activity* WAU.gFW <sup>-1</sup> (%)
<i>T. sinensis</i>	42.52 ± 6.23	26.38 ± 3.81	16.14 (37.96)
<i>B. convoluta</i>	63.78 ± 7.49	37.47 ± 4.28	26.31 (41.25)

Values are expressed as means ± SD, n = 6.

\* The value of extracellular CA activity is the difference of the means of total CA activity and that of CA activity with AZ. Number inside parentheses are percentages relative to the total CA activity.

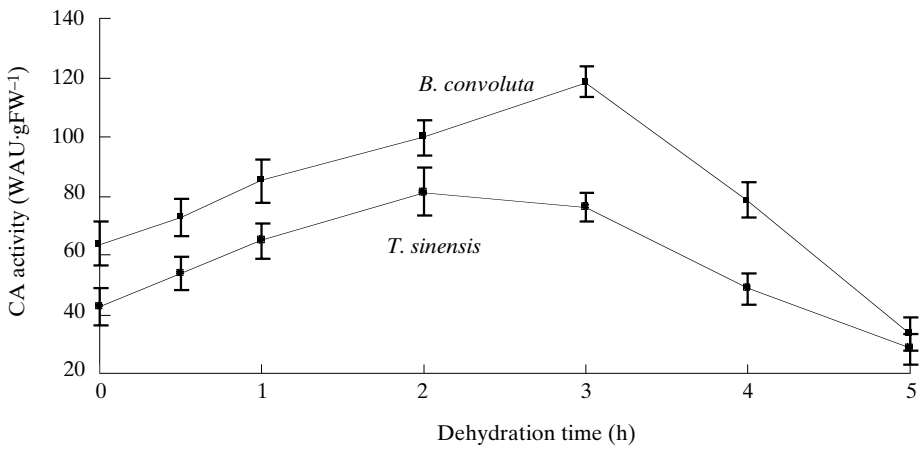


Fig. 2. The activity of carbonic anhydrase ( $M \pm SD$ ,  $n = 6$ ) of *T. sinensis* and *B. convoluta* at different dehydration times.

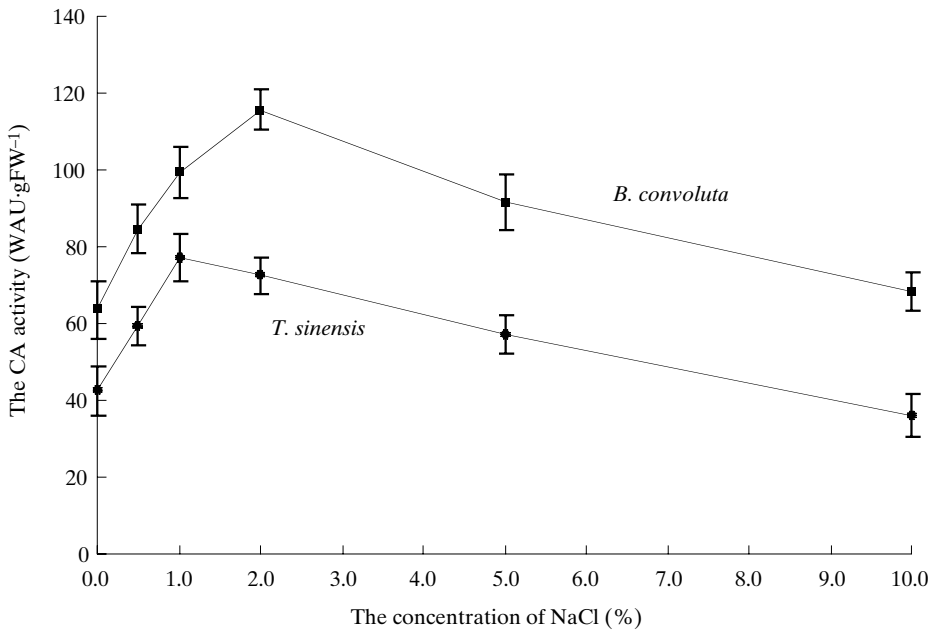


Fig. 3. The activity of carbonic anhydrase ( $M \pm SD$ ,  $n = 6$ ) of *T. sinensis* and *B. convoluta* at different concentrations of sodium chloride.

Often time moss photosynthesis does not decrease immediately upon dehydration (Mayaba & Beckett, 2003). This may be related again to the great increase in activity of CA in mosses after a period of dehydration. The increase of the CA activity enhances the conversion of bicarbonate into  $H_2O$  and  $CO_2$ . There is evidence supporting this interpretation since a significant correlation

between CA activity and net photosynthetic rate has been found in such plants as tobacco and *Brassica juncea* (Williams *et al.*, 1996; Ahmad *et al.*, 2001) (see also Fig. 2).

Sodium chloride used in this study seems to perform two functions, one as an osmotic agent, and another, as CA inhibitor. Salt stress induced by an osmotic agent may cause the increase of water potential in the plant cell to induce dehydration. The activity of CA in mosses gets higher when the concentration of sodium chloride is at 2%, although a much higher concentration of sodium chloride restrains the CA activity (Johansson & Forsman, 1994). Our experiments show that the effected increase of CA activity induced by the dehydration of salt is greater than that of the decrease of CA activity induced by the inhibitory ions when the concentration of sodium chloride is less than 2% (see Fig. 3).

According to the analysis above, it seems that the following conclusion can be drawn: *T. sinensis* and *B. convoluta* can grow on limestone beds because they can absorb a large amount of water and bicarbonate after rain (Eldridge & Tozer, 1997; Zhang *et al.*, 2002). However, when *T. sinensis* and *B. convoluta* suffer from water stress because of drought, the CA activities can get higher to provide the mosses with water by converting the bicarbonate into water and CO<sub>2</sub>.

**Acknowledgements.** This work was supported by grant no. 40273038 of National Natural Science Foundation of China and no.2001-6-3 of Karst Dynamic Laboratory, Ministry of Land and Resources, P.R.C. We are grateful to Professor Benito C. Tan and anonymous reviewers for valuable comments and suggestions on the manuscript.

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