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SHORT REPORTS

CYCLIC ADENOSINE MONOPHOSPHATE IN FRUITS OF *ZIZYPHUS JUJUBA*

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Key Word Index *Zizyphus jujuba*; Rhamnaceae; cyclic adenosine-monophosphate; cyclic AMP.

Abstract—High levels of cyclic AMP activity were detected in the fruit of *Zizyphus jujuba*. The partially purified cyclic AMP-like substance was found in amounts ranging from 100 to 150 nmol/g (fr. wt) by both a competitive binding assay and radioimmunoassay. The cyclic AMP-like substance also showed the same elution pattern as authentic cyclic AMP and was decomposed by cyclic nucleotide-specific phosphodiesterase.

Cyclic adenosine monophosphate (cyclic AMP) has been established as an intracellular second messenger in the action of various hormones, prostaglandins and catecholamines [1]. There is considerable evidence to suggest that the cyclic nucleotide system plays a significant role in the pathophysiology of bronchial asthma and allergic diseases [1]. Ancient Chinese medical prescriptions which were used to treat these diseases have been described in books of Chinese medicine [2, 3] and tests were therefore performed on such plants to assay for the possible presence of cyclic AMP.

Water extracts of 180 different plants were tested for cyclic AMP activity by a competitive binding assay. Of these, only the fruit of *Zizyphus jujaba* was positive. The active substance was eluted by methods known to isolate cyclic AMP. After incubation with cyclic nucleotide-specific phosphodiesterase (EC 3.1.4.17) at 37° for 60 min, more than 98% of the activity disappeared and 5′-AMP was detected by TLC. These results suggest that the isolated substance is cyclic AMP.

Cyclic AMP levels in matured dry fruit of *Z. jujuba* range from 100 to 600 nmol/g (dry wt) and in matured fresh fruit 100 to 150 nmol/g (fr wt) as measured by both competitive binding assay and radioimmunoassay. Moreover, both methods usually gave the same value on a given sample.

There have been a few reports of the existence of cyclic AMP-like compounds in higher plants [4–7]. However, the amounts reported are at most 10 nmol (dry wt). The levels of cyclic AMP in fresh fruit of *Z. jujuba* are therefore the highest yet found, not only in plants but also in animal tissue [8]. Only trace amounts of the cyclic AMP-like substance (20–25 pmol/g fr wt) were detected in fresh immature fruit of *Z. jujuba*. This suggests that an increase in cyclic AMP synthesis and/or decrease in cyclic AMP catabolism occurs during maturation of the fruit.

The cyclic AMP-like substance, purified using Dowex AG 1 × 4 (Cl-) and Alumina Woelm N-Super 1, showed the same physicochemical properties as authentic cyclic AMP by TLC using different solvents, by UV spectroscopy, and by HPLC [9].

EXPERIMENTAL

Fruit of *Z. jujuba* and other plants was purchased from Uchida Wakanyaku Company, Tokyo, Japan, who imported them from China. Fresh fruit of *Z. jujuba* was also obtained from Kyoto Takeda Harbal Garden, Kyoto, Japan.

Assay. Samples were diluted with 50 mM NaOAc buffer, pH 4 and assayed by both competitive binding [10] and radioimmunoassay [11]. Kits for these assays were purchased from Boehringer and Yamasa, respectively.

Extraction. One hundred and eighty different plants known to Chinese medicine [2, 3] were screened for cyclic AMP activity. Dried plant material (10 g) was suspended in 300 ml of H_2O and boiled until the vol. reached 100 ml (*ca* 40 min). Authentic cyclic AMP was found to be stable during this procedure. The soln was centrifuged at 25 000 *g* for 30 min and the supernate passed through a membrane filter. These H_2O extracts were serially diluted with 50 mM NaOAc buffer, pH 4 and cyclic AMP activity was determined. Only the fruit of *Z. jujuba* was positive for cyclic AMP activity in the screening procedure described above. We isolated cyclic AMP-like substance as follows: dried crushed *Z. jujuba* fruit (100 g) was suspended in 500 ml H_2O and boiled for 30 min. The solid material was collected on a stainless steel mesh (100 squares/cm) and extracted two more times with H_2O in the same way. The resultant extracts were pooled, filtered through sintered glass, lyophilized and resuspended in 500 ml 95% EtOH.

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Extraction with EtOH was carried out by heating to 80° for 3 hr. The resultant extract was filtered through sintered glass, lyophilized, redissolved in 100 ml 50 mM NaOAc buffer, pH 4. One ml of this soln was passed through a column of Dowex AG 1 × 8, 0.6 × 3.0 cm, (formate form, 200–400 mesh) according to ref. [12]. The resin was washed with 10 ml H_2O, and > 90% of the cyclic AMP-like substance was eluted with 10 ml 2 N HCO_2H. [^{3}H]-Cyclic AMP was eluted identically when added to the plant extract starting material.

Sensitivity to phosphodiesterase. The partially purified fraction from the Dowex AG 1 × 8 column was incubated at 37° for 60 min with cyclic nucleotide-specific phosphodiesterase from beef heart (EC 3.1.4.17) (Boehringer) in the presence of 5 mM $MgCl_2$ at pH 8.6. The reaction was stopped by heating to 100° for 2 min. Almost 98% of the cyclic AMP activity was lost and generation of 5′-AMP was detected by TLC (Si gel) in C_6H_6–EtOAc–MeOH (1:1:3). These results indicate that the structure of this substance is probably the same as that of cyclic AMP from animal tissue [13, 14].

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REFERENCES

1. Kaliner, M. (1977) *J. Clin. Invest.* **60**, 951.
2. Cyong, J., Hanabusa, K. and Otsuka, Y. (1979) *Proc. Wakanyaku Sympos.*, in press.
3. Otsuka, K. and Yakazu, D. (1973) *Keiken-shyoho-bunryoshu.* Idono-nihon, Tokyo.
4. Brown, E. G. and Newton, R. P. (1973) *Phytochemistry* **12**, 2683.
5. Raymond, P., Marayman, A. and Pradet, A (1973) *Biochem. Biophys. Res. Commun.* **53**, 1115.
6. Lin, P. P.-C. (1974) *Adv. Cyclic Nucleotide Res.* **4**, 439.
7. Brown, E. G., Al-Najafi, T. and Newton, R. P. (1979) *Phytochemistry* **18**, 9.
8. Steiner, A. L., Kipnis, D. M., Utiger, R. and Parker, C. W. (1969) *Proc. Natl. Acad. Sci.* U.S.A. **64**, 367.
9. Hanabusa, K. and Cyong, J. (1980) *Planta Med.* (submitted).
10. Cyong, J. and Okada, H (1975) *Methods Immunol.* **4**, 1183.
11. Steiner, A. L., Parker, C. W. and Kipnis, D. M. (1972) *J. Biol. Chem.* **247**, 1106.
12. Kuo, J. F. and Greengard, P. (1970) *J. Biol. Chem.* **245**, 2493.
13. Nair, K. G. (1966) *Biochemistry* **5**, 150.
14. Appleman, M. M., Thompson, W. J. and Russell, T. R. (1973) *Adv. Cyclic Nucleotide Res.* **3**, 65.