Biochemical Pharmacology, Vol. 51, pp. 339–343, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
SSDI 0006-2952(95)02188-4

Specific Inhibition of Na^+,K^+-ATPase Activity by Atractylon, a Major Component of Byaku-jutsu, by Interaction with Enzyme in the E_2 State

Kanako Satoh, Fumiko Nagai, Keiko Ushiyama and Itsu Kano*

DEPARTMENT OF TOXICOLOGY, THE TOKYO METROPOLITAN RESEARCH LABORATORY OF PUBLIC HEALTH, TOKYO 169, JAPAN

ABSTRACT. Atractylon, a major component of the crude drug "Byaku-jutsu" (rhizomes of *Atractylodes japonica*), strongly inhibited Na^+,K^+-ATPase activity with an I_{50} value of 8.9×10^{-6} M. It also inhibited Mg^{2+}-ATPase, H^+,K^+-ATPase, H^+-ATPase and Ca^{2+}-ATPase activities, but less potently. No effects on alkaline and acid phosphatase activities were observed. The inhibition of Na^+,K^+-ATPase activity by atractylon was noncompetitive with respect to ATP and was greater with increasing K^+ concentration, whereas it was not affected by Na^+ concentration. The activity of K^+-dependent *p*-nitrophenyl phosphatase, a partial reaction of Na^+,K^+-ATPase, was inhibited noncompetitively with respect to substrate (I_{50} value of 1.8×10^{-5} M), and the inhibition rate was independent of the K^+ concentration. Furthermore, atractylon increased the K_i value for Na^+ from 130 to 190 mM, but did not alter the K_i value for ATP. Inhibition of the phosphoenzyme formation by atractylon was greater at 0.1 M than at 1 M NaCl. K^+-dependent dephosphorylation (E_2-P to $K \cdot E_2$) was inhibited by atractylon, whereas ADP-sensitive ($Na \cdot E_1$-P to $Na \cdot E_1$) and non-specific dephosphorylation steps were not affected. These results suggest that atractylon, a specific inhibitor of Na^+,K^+-ATPase, interacts with enzyme in the E_2 state and inhibits the reaction step from E_2-P to $K \cdot E_2$. BIOCHEM PHARMACOL 51;3:339–343, 1996.

KEY WORDS. Na^+,K^+-ATPase; atractylon; Byaku-jutsu; rhizomes of *Atractylodes japonica*; K^+-pNPPase

"Byaku-jutsu" (rhizomes of *Atractylodes japonica*) is an important traditional Chinese drug used for the treatment of water retention in the body. Administration of the decoction of Byaku-jutsu causes diuresis in humans [1], and its alcohol extract has a diuretic effect in mice [2]. Previously, we found that the ethanol extract of Byaku-jutsu strongly inhibits Na^+,K^+-ATPase activity (I_{50} value of 15.2 μg/mL) [3]. Byaku-jutsu contains 1.5 to 3.0% (w/w) sesquiterpenoids, such as atractylon (Fig. 1), atractylenolide III, 3β-hydroxyatractylon and 3β-acetoxyatractylon, as well as polyacetylene compounds such as TDDM,† (6*E*,12*E*)-tetradecadiene-8,10-diyne-1,3-diol and 6-methyl-2-geranyl-*p*-benzoquinone [4–8]. We found that Na^+,K^+-ATPase activity is inhibited by atractylenolide III and TDDM with I_{50} values of 7.0×10^{-4} and 1.7×10^{-4} M, respectively [8]. Atractylon, which accounts for 20% of the sesquiterpenoids [4], was reported to prevent hepatotoxicity due to $CuCl_2$ in rats [9]. Furthermore, it has anti-microbial activity, anti-inflammatory activity [10], and anti-ulcer action [11]. Atractylon was found in the present work to be the strongest inhibitor of Na^+,K^+-ATPase among the constituents of Byaku-jutsu that we examined, and the mechanism of its action was investigated.

MATERIALS AND METHODS

The crude membrane fraction and purified Na^+,K^+-ATPase (SDS-enzyme) of horse kidney [12], and rat gastric membrane vesicles were prepared according to methods described previously [13]. Horse and rat brain membrane fractions were prepared according to Sweadner [14]. Atractylon (98% pure) was purchased from the Yoneyama Yakuhin Industries Co. (Osaka, Japan). EDTA, EGTA, and CDTA were obtained from the Dojindo Chemical Co. (Kumamoto, Japan). Other reagents were of the highest grade commercially available.

Quantification of Atractylon

The stability of atractylon in the reaction mixture was examined by HPLC analysis and UV absorbance measurements (UV-250, Shimadzu). HPLC was carried out with a column of TSK-GEL ODS-80T_M (TOSOH) with acetonitrile:water (8:2) at a flow rate of 1 mL/min. The absorbance of the peaks at 219 nm was monitored to quantify atractylon.

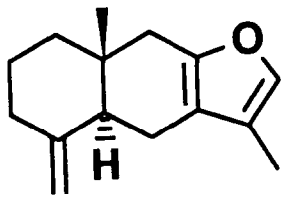
Assay of Enzyme Activities

Na^+,K^+-ATPase (ouabain-sensitive) activity was determined essentially according to a method reported previously [15], and

* Corresponding author: Dr. Kanako Satoh, Department of Toxicology, The Tokyo Metropolitan Research Laboratory of Public Health, 3-24-1, Hyakunin-cho, Shinjuku-ku, Tokyo 169, Japan. Tel. 3-3363-3231, Ext. 425; FAX 3-3368-4060.

† *Abbreviations:* TDDM, (6*E*,12*E*)-tetradecadiene-8,10-diyne-1,3-diol monoacetate; K^+-pNPPase, K^+-dependent *p*-nitrophenyl phosphatase; and CDTA, *trans*-1,2-cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid.

Received 17 April 1995; accepted 15 August 1995.



Atractylon

FIG. 1. Structure of atractylon.

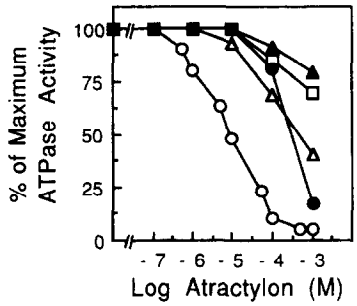


FIG. 2. Effect of atractylon on ATPase activities. Enzyme activities were determined in the presence of various concentrations of atractylon. ATPase activities without atractylon were taken as 100%, and were 39.8, 0.23, 0.04, 0.11 and 0.04 μmol Pi/mg protein/min for Na^+,K^+-ATPase (○) in SDS-enzyme, Mg^{2+}-ATPase (△), H^+-ATPase (▲) and Ca^{2+}-ATPase (●) in horse kidney crude membrane fraction, and H^+,K^+-ATPase (□) in rat gastric membrane vesicles, respectively. The SD was always less than 2.5% (N = 6).

Mg^{2+}-ATPase activity was estimated as ouabain-insensitive activity [16]. Atractylon was dissolved in ethanol:DMSO (8:2, v/v). An aliquot of the solution (10 μL) was added to the reaction mixture (0.5 mL) containing SDS-enzyme (1 μg protein/mL) or crude membrane fraction (100 μg protein/mL), 3 mM ATP, 140 mM NaCl, 14 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM EGTA, and 50 mM imidazole–HCl buffer (pH 7.2) with or without 0.5 mM ouabain. The activity of SDS-enzyme was inhibited almost completely by 1.0×10^{-5} M ouabain. The concentrations of ethanol and DMSO (1.6 and 0.4%, respectively) in the reaction mixture had no effect on the enzyme activities. Mg^{2+}-, H^+- and Ca^{2+}-ATPase activities were determined with horse kidney crude membrane fraction. The enzyme for H^+-ATPase activity was sonicated just before incubation with a sonifier (Branson B-12). The reaction mixture (0.5 mL) for H^+-ATPase contained 5 mM ATP, 20 mM phosphoenol pyruvic acid, 32 μg pyruvate kinase, 5 mM $MgSO_4$ and Tris–H_2SO_4 buffer (pH 7.4) [17] and that for Ca^{2+}-ATPase activity contained 3 mM ATP, 5 mM $MgCl_2$, 140 mM NaCl, 14 mM KCl, 0.1 mM ouabain, 0.5 mM EGTA, 0.8 mM $CaCl_2$ and 30 mM imidazole–HCl buffer (pH 7.4) [18]. H^+,K^+-ATPase was determined by rat gastric membrane vesicles (100 μg protein/mL) in a reaction mixture (0.5 mL) containing 1 mM ATP, 2 mM $MgCl_2$ and 20 mM Tris–HCl buffer (pH 6.8) with or without 10 mM KCl [13]. Alkaline phosphatase [19] and acid phosphatase [20] were measured as determined previously. K^+-pNPPase activity was determined in a reaction mixture (0.5 mL) containing SDS-enzyme (1 μg protein/mL), 20 mM pNPP, 15 mM KCl, 10 mM $MgCl_2$, 0.1 M Tris–HCl buffer (pH 7.7) and 10 μL of atractylon solution at 37° for 10 min [21]. Phosphorylation and dephosphorylation of the SDS-enzyme were carried out according to a previous report [16].

RESULTS

Effects of Atractylon on ATPase and Phosphatase Activities

The inhibition of Na^+,K^+-ATPase activity by atractylon was detected at a concentration as low as 5.0×10^{-7} M, and was concentration dependent (Fig. 2). At 5.0×10^{-4} M, atractylon caused more than 95% inhibition of the activity (Fig. 2). The apparent I_{50} value of atractylon for Na^+,K^+-ATPase from purified horse kidney enzyme was 8.9×10^{-6} M, which was about the same as with rat brain enzyme from membrane fraction. Moreover, the inhibition curves of these two enzyme preparations coincided exactly (data not shown). In our experiments, we did not find any difference between horse kidney and rat brain in terms of I_{50} values and inhibition curves.

Atractylon at a concentration of 8.9×10^{-6} M (I_{50} value for Na^+,K^+-ATPase) inhibited Mg^{2+}-ATPase activity by only 8%, and no inhibition of H^+,K^+-ATPase, Ca^{2+}-ATPase or H^+-ATPase was observed (Fig. 2). The I_{50} values of 4.4×10^{-4} M for Mg^{2+}-ATPase and of 3.5×10^{-4} M for Ca^{2+}-ATPase were much greater than the value for Na^+,K^+-ATPase. The extents of inhibition of H^+,K^+-ATPase and H^+-ATPase activities were only 30 and 20%, respectively, when the concentration of atractylon was raised to 1.0×10^{-3} M (Fig. 2). Furthermore, atractylon had no effect on alkaline and acid phosphatase activities (data not shown). These results demonstrate that atractylon predominantly inhibited Na^+,K^+-ATPase activity among the phosphatases examined.

Interaction of Atractylon and Na^+,K^+-ATPase

The assay of Na^+,K^+-ATPase activity was started by the addition of SDS-enzyme and ATP to the reaction mixture containing 8.9×10^{-6} M atractylon that had been preincubated at 37° (Table 1). The preincubation of atractylon in the presence of ATP was also examined (Table 1). The inhibition by atractylon was 50% without preincubation in each case, but was decreased to 22% and less than 2% by preincubation for 10 and 45 min, respectively. The inhibitory effect remained constant at 50%, however, when the assay of enzyme activity was started by adding ATP after preincubation of atractylon with SDS-enzyme for up to 45 min (Table 1). These results indicated that atractylon was unstable when the enzyme was not present in the reaction mixture. HPLC analysis showed that the amount of atractylon was decreased to less than 1% after a 45-min incubation in the reaction mixture at 37°.

The inhibition of Na^+,K^+-ATPase activity by atractylon was completely reversible, i.e. the activity returned to the control value when atractylon was removed by centrifugation following dilution of the reaction mixture.

TABLE 1. Effects of preincubation of atractylon on the activity of Na^+,K^+-ATPase

Preincubation Condition	Time (min)	Inhibition (%)
Atractylon	0	50.2 ± 1.0
	10	22.4 ± 0.4
	45	<2
Atractylon + ATP	0	50.4 ± 1.1
	10	22.2 ± 0.4
	45	<2
Atractylon + enzyme	0	50.2 ± 1.0
	10	49.7 ± 0.9
	45	50.4 ± 1.0

Preincubation of atractylon (8.9×10^{-6} M) at 37° was performed under various conditions. Then, the reaction of Na^+,K^+-ATPase was started by adding SDS-enzyme plus ATP, SDS-enzyme, and ATP, respectively. Values are means ± SD ($N = 7$). The basal value for Na^+,K^+-ATPase activity was 32.5 μmol P_i/mg/min.

Effects of Atractylon on Na^+,K^+-ATPase Activity in the Presence of Various Ligands

Na^+,K^+-ATPase activity was assayed in the presence of atractylon and various concentrations of ATP in the reaction mixture, and the mode of inhibition was found to be noncompetitive with respect to ATP.

Na^+,K^+-ATPase activity was assayed in a reaction mixture containing various concentrations of K^+ or Na^+ with or without 8.9×10^{-6} M atractylon (Fig. 3). Atractylon had no effect on ATPase activity in the absence of K^+. The inhibition by atractylon was small at low concentrations of K^+ (4% at 0.3 mM K^+). However, it gradually increased as the K^+ concentration was raised, and at concentrations above 3.8 mM it was almost constant (50%). On the other hand, the extent of inhibition by atractylon (8.9×10^{-6} M) was constant at 50% irrespective of the Na^+ concentration in the range of 0 to 200 mM.

The inhibitory effect of ouabain, a specific inhibitor of Na^+,K^+-ATPase activity, was examined in the presence of 8.9×10^{-6} M atractylon. The I_{50} value (3.9×10^{-7} M) of ouabain for the SDS-enzyme was the same regardless of the presence or the absence of atractylon. Furthermore, the I_{50} value of atractylon was not altered by the addition of 3.9×10^{-7} M ouabain.

Effects of Atractylon on K^+-pNPPase Activity

K^+-pNPPase activity, which reflects reaction in the E_2 state of Na^+,K^+-ATPase [22], was inhibited by atractylon in a concentration-dependent manner, with complete inhibition by 4.0×10^{-4} M (Fig. 4). The apparent I_{50} value was 1.8×10^{-5} M. The mode of inhibition appeared to be noncompetitive with respect to pNPP (data not shown). The I_{50} value of ouabain for K^+-pNPPase activity (5.1×10^{-6} M) was not altered by the addition of atractylon (data not shown).

The inhibition rate by atractylon was independent of K^+ concentration (Fig. 5a). However, K^+-pNPPase activity was decreased with an increase of Na^+ concentration from 6.3 to 500 mM; the K_i value for Na^+ obtained from a Hill plot was 130 mM. In the presence of 1.8×10^{-5} M atractylon (I_{50}), the inhibitory effect of Na^+ was less, and the K_i value for Na^+ was 190 mM (Fig. 5b).

Although K^+-pNPPase activity was inhibited by ATP, the inhibition rate was almost the same in the presence and absence of 1.8×10^{-5} M atractylon (data not shown).

Effects of Atractylon on Phosphorylation and Dephosphorylation of the Enzyme

The effect of atractylon on phosphoenzyme formation, which is dependent on ATP, Na^+, and Mg^{2+}, was examined at steady state (Fig. 6). Phosphoenzyme formation was decreased with increasing concentrations of atractylon. The inhibition by atractylon was greater in 0.1 M than in 1 M NaCl, with I_{50} values of 2.0×10^{-5} and 1.0×10^{-3} M, respectively.

Dephosphorylation was carried out in the presence of atractylon. The phosphoenzyme formed in 0.1 M NaCl at 0° for 10 sec was chased with CDTA (non-specific dephosphorylation), CDTA + ADP (ADP-sensitive dephosphorylation), or CDTA + KCl (K^+-dependent dephosphorylation) at 0° for 5 sec (Fig. 7). Non-specific or ADP-sensitive dephosphorylation was not affected by the addition of atractylon. The phosphoenzyme was decreased to an undetectable level after the chase with CDTA + 1 mM KCl in the absence of atractylon. When the

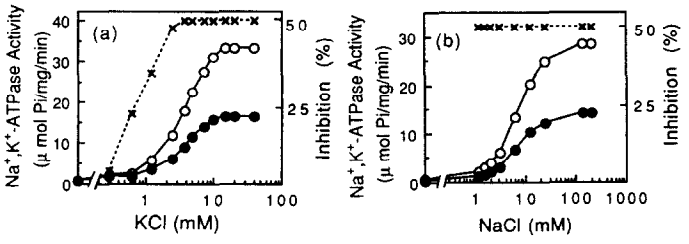


FIG. 3. Effect of K^+ or Na^+ concentration on Na^+,K^+-ATPase activity in the presence of atractylon. The SDS-enzyme was incubated with (●) or without (○) atractylon (8.9×10^{-6} M). (a) The concentration of NaCl was fixed at 140 mM and that of KCl was varied. (b) The concentration of KCl was fixed at 14 mM and that of NaCl was varied. The percent inhibition caused by atractylon at each point is indicated (x). The SD was always less than 2.4% (N = 5).

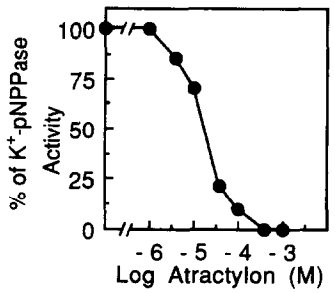


FIG. 4. Inhibition of K^+-pNPPase activity by atractylon. Enzyme activity was determined in the presence of various concentrations of atractylon. The activity without atractylon was taken as 100% (6.1 μmol/mg protein/min). The SD was always less than 1.9% (N = 4).

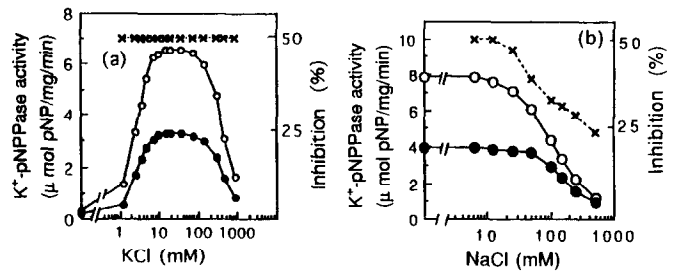


FIG. 5. Effect of atractylon on K^+-pNPPase activity in the presence of various concentrations of K^+ or Na^+. Enzyme activity was determined with (●) or without (○) atractylon (1.8×10^{-5} M). (a) The concentration of KCl was varied. (b) The concentration of KCl was fixed at 15 mM and that of NaCl was varied. The percent inhibition caused by atractylon at each point is indicated (x). The SD was always less than 2.2% (N = 5).

phosphoenzyme was chased with CDTA + 0.5 mM KCl, the remaining phosphoenzyme level was 7% of the initial phosphoenzyme level in the absence of atractylon, and the corresponding values were 15, 40 and 73% in the presence of 1.0×10^{-6}, 1.0×10^{-5} and 1.0×10^{-3} M atractylon, respectively. Thus atractylon inhibited the K^+-dependent dephosphorylation in 0.1 M NaCl. On the other hand, in 1 M NaCl, nonspecific, ADP-sensitive and K^+-dependent dephosphorylations were not altered notably by the addition of atractylon (data not shown).

DISCUSSION

Atractylon was found to be a specific inhibitor of Na^+,K^+-ATPase ($I_{50} = 8.9 \times 10^{-6}$ M) on the basis of its effects on a group of phosphatase activities (Na^+,K^+-ATPase, Mg^{2+}-ATPase, Ca^{2+}-ATPase, H^+,K^+-ATPase, H^+-ATPase, and alkaline and acid phosphatases). The results imply that the inhibitory effect of Na^+,K^+-ATPase in Byaku-jutsu is attributable mainly to atractylon, not atractylenolide III or TDDM.

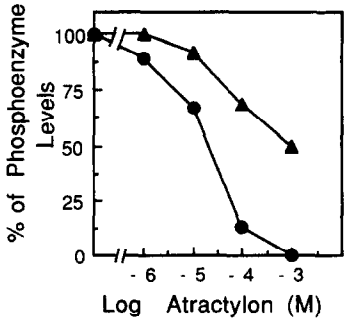


FIG. 6. Effect of atractylon on the phosphorylation of Na^+,K^+-ATPase. The SDS-enzyme (sp. act. 46 μmol P_i/mg protein/min) was phosphorylated with 1 μM [γ-^{32}P]ATP for 10 sec at 0° in a solution of 2 mM $MgCl_2$, 20 mM histidine–HCl buffer, pH 7.5, and 1 or 0.1 M NaCl in the presence of various concentrations of atractylon. The phosphoenzyme levels without atractylon in 1 M (▲) and 0.1 M (●) NaCl, 30.3 nmol/mg protein and 45.5 nmol/mg protein, respectively, were taken as 100%. The SD was always less than 4.6% (N = 4).

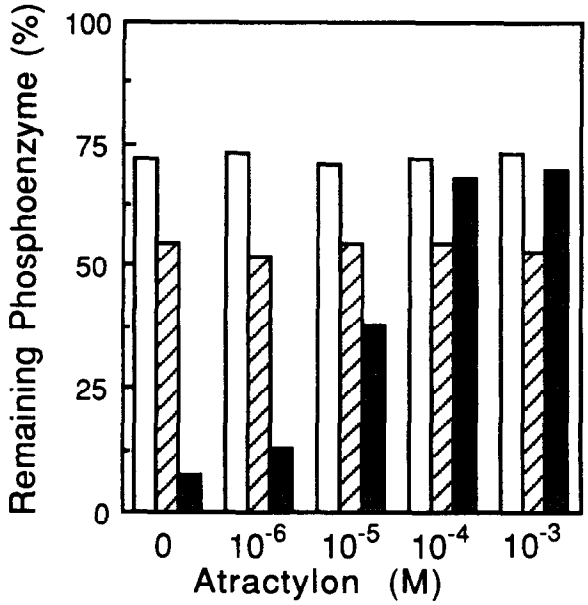
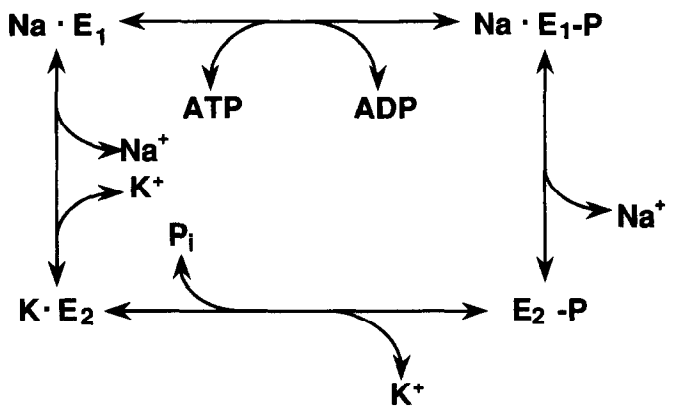


FIG. 7. Effect of atractylon on the dephosphorylation of Na^+,K^+-ATPase. The dephosphorylation of phosphoenzyme formed in 0.1 M NaCl without atractylon (Fig. 6) was performed for 5 sec at 0° in the presence of 20 mM CDTA + 0.5 mM KCl (■), 20 mM CDTA + 5 mM ADP (▨) or 20 mM CDTA (□) with various concentrations of atractylon. Values are given as percentages relative to the initial phosphoenzyme level (100%) formed without atractylon as described in the legend to Fig. 6. The SD was always less than 3.7% (N = 4).

In the reaction of Na^+,K^+-ATPase, the conformation of the enzyme changes from $K \cdot E_2$ to $Na \cdot E_1$ when the Na^+ concentration reaches a certain level, and $Na \cdot E_1$ is phosphorylated by ATP to afford $Na \cdot E_1$-P. The $Na \cdot E_1$-P converts to E_2-P and dephosphorylates to $K \cdot E_2$ when the K^+ concentration reaches a certain level (Scheme 1) [23, 24]. The inhibition by atractylon of Na^+,K^+-ATPase increased with an increase of the K^+ concentration, but the extent of inhibition was not affected by Na^+ concentration. The inhibition of K^+-pNPPase was independent of the concentration of K^+. On the other hand, the K_i value for Na^+ was decreased by the addition of atractylon.



SCHEME 1.

Therefore, atractylon may interact with the enzyme in the E_2 state to inhibit the Na^+,K^+-ATPase activity.

When the phosphorylation reaction by ATP is carried out in 0.1 M NaCl, $Na \cdot E_1$ is converted to E_2-P via $Na \cdot E_1$-P, for the most part [25, 26]. On the other hand, the phosphoenzyme formed in 1 M NaCl is mainly $Na \cdot E_1$-P [25, 26]. Our results indicate that the inhibition of E_2-P formation in 0.1 M NaCl by atractylon was greater than that of E_1-P formation in 1 M NaCl. However, atractylon inhibited K^+-dependent dephosphorylation (from E_2-P to $K \cdot E_2$). We presume that the blocking of the K^+-dependent dephosphorylation process (from E_2-P to $K \cdot E_2$) ultimately leads to the inhibition of $E \cdot P$ formation.

The effects of atractylon on the overall and partial reactions of Na^+,K^+-ATPase support the idea that atractylon interacts with E_2-P and inhibits the reaction step from E_2-P to $K \cdot E_2$. However, the binding site of atractylon was considered to be different from that of ouabain, which also inhibits Na^+,K^+-ATPase activity by binding to the enzyme in the E_2 state [23].

We previously elucidated the inhibition mechanism of Na^+,K^+-ATPase by β-eudesmol. β-Eudesmol, a major component of So-jutsu, is a specific inhibitor of Na^+,K^+-ATPase activity (I_{50} value of 1.6×10^{-4} M) and prevents the transition from $Na \cdot E_1$ to $Na \cdot E_1$-P [16, 21]. Atractylon and β-eudesmol should be useful inhibitor probes, since they interact with the enzyme in different states. It would be interesting to identify the receptor sites at the structural level.

The inhibition of Na^+,K^+-ATPase activity by atractylon causing the suppression of reabsorption of water and Na^+ in the kidneys could explain at least one of the diuretic effects of Byaku-jutsu in humans and mice. In Chinese traditional medicine, a crude drug is not used independently, but rather is prescribed as part of a combination. We intend to examine the effects of combinations of atractylon and other constituents of Byaku-jutsu with components of other Chinese medicines used with Byaku-jutsu in order to obtain insight into the effects of the combined prescriptions.

We thank Dr. T. Seto and Dr. I. Yasuda at the Department of Pharmaceutical Sciences, The Tokyo Metropolitan Research Laboratory of Public Health, for advice on the stability of atractylon and the components of Byaku-jutsu.

References

1. Utizumi S and Sakaguti H, The study on diuretic crude drugs. *Nippon Toyo Igaku Zasshi* **4:** 1–7, 1953.
2. Tsurumi K, Taki K, Ichioka H, Esaki S, Sakai S and Sawazaki S, On the diuretic action of crude drugs. II. Alcohol extracts. *Gifu Prefec Med School Bull* **11:** 138–142, 1963.
3. Satoh K, Yasuda I, Nagai F, Ushiyama K, Akiyama K and Kano I, The effects of crude drugs using diuretic on horse kidney ($Na^+ + K^+$)-adenosine triphosphatase. *Yakugaku Zasshi* **111:** 138–145, 1991.
4. Hikino H, Hikino Y and Yosioka I, Structure and autoxidation of atractylon. *Chem Pharm Bull (Tokyo)* **10:** 641–642, 1962.
5. Nishikawa Y, Yasuda I, Watanabe Y and Seto T, Studies on the evaluation of crude drugs. II. Identification of the ingredients of *Atractylodes* by thin-layer chromatography, gas chromatography and gas chromatography mass spectrometry, and the physical and chemical evaluation. *Shoyakugaku Zasshi* **30:** 132–137, 1976.
6. Pachaly P, Lansing A and Sin KS, Inhaltsstoffe von *Atractylis Koreana*. *Planta Med* **55:** 59–61, 1989.
7. Sakurai T, Yamada H, Saito K and Kano Y, Enzyme inhibitory activities of acetylene and sesquiterpene compounds in Atractylodes Rhizome. *Biol Pharm Bull* **16:** 142–145, 1993.
8. Yasuda I, Takano I, Akiyama K, Satoh K, Nagai F, Ushiyama K and Kano I, The inhibitory effects of Atractylodes Rhizoma on kidney Na^+,K^+-ATPase and its inhibitory constituents. *J Med Pharm Soc Wakan-Yaku* **7:** 420–421, 1990.
9. Kiso Y, Tohkin M and Hikino H, Mechanism of antihepatotoxic activity of atractylon. I: Effect on free radical generation and lipid peroxidation. *Planta Med* **2:** 97–100, 1985.
10. Sin KS, Kim HP, Lee WC and Pachaly P, Pharmacological activities of the constituents of Atractylodes Rhizomes. *Arch Pharm Res* **12:** 236–238, 1989.
11. Matsuda H, Li Y, Taniguchi K, Yamahara J and Tamai Y, Imaging analysis of antiulcer action and the active constituent of Atractylodis Rhizoma. *Yakugaku Zasshi* **111:** 36–39, 1991.
12. Nakao T, Ohno-Fujitani T and Nakao M, Sodium and potassium ion-dependent change in oligomerization of Na,K-ATPase in $C_{12}E_8$ detected by low-angle laser light scattering technique in combination with high performance porous silica-gel chromatography. *J Biochem (Tokyo)* **94:** 689–697, 1983.
13. Maeda M, Tagaya M and Futai M, Modification of gastric ($H^+ + K^+$)-ATPase with pyridoxal 5′-phosphate. *J Biol Chem* **263:** 3652–3656, 1988.
14. Sweadner KJ, Preparation of the α(+) isozyme of the Na^+,K^+-ATPase from mammalian axolemma. *Methods Enzymol* **156:** 65–71, 1988.
15. Satoh K, Nakao T, Nagai F, Kano I, Nakagawa A, Ushiyama K, Urayama O, Hara Y and Nakao M, A monoclonal antibody against horse kidney ($Na^+ + K^+$)-ATPase inhibits sodium pump and E_2K to E_1 conversion of ($Na^+ + K^+$)-ATPase from outside of the cell membrane. *Biochim Biophys Acta* **994:** 104–113, 1989.
16. Satoh K, Nagai F, Ushiyama K, Yasuda I, Akiyama K and Kano I, Inhibition of Na^+,K^+-ATPase activity by β-eudesmol, a major component of Atractylodis Lanceae Rhizoma, due to the interaction with enzyme in the $Na \cdot E_1$ state. *Biochem Pharmacol* **44:** 373–378, 1992.
17. Smith AL, Preparation, properties, and conditions for assay of mitochondria: Slaughterhouse material, small scale. *Methods Enzymol* **10:** 81–86, 1967.
18. Nagai F, Ushiyama K, Kano I, Nakagawa A, Nakao T and Nakajima A, Inhibition of calmodulin stimulation of phosphodiesterase and Ca^{2+},Mg^{2+}-ATPase activities and shape change of erythrocyte ghosts by chloroquine. *Biochem Pharmacol* **36:** 3433–3437, 1987.
19. Fernley HN, Mammalian alkaline phosphatases. In: *The Enzymes* (Ed. Boyer PD), Vol. 4, pp. 417–447. Academic Press, New York, 1971.
20. Hollander VP, Acid phosphatases. In: *The Enzymes* (Ed. Boyer PD), Vol. 4, pp. 449–498. Academic Press, New York, 1971.
21. Satoh K, Nagai F and Kano I, Interaction of β-eudesmol with Na^+,K^+-ATPase: Inhibition of K^+-pNPPase. *Biochem Pharmacol* **44:** 993–995, 1992.
22. Nagai K and Yoshida H, Biphasic effects of nucleotides on potassium-dependent phosphatase. *Biochim Biophys Acta* **128:** 410–412, 1966.
23. Post RL, Kume S, Tobin T, Orcutt B and Sen AK, Flexibility of an active center in sodium-plus-potassium adenosine triphosphatase. *J Gen Physiol* **54:** 306s–326s, 1969.
24. Taniguchi K and Post RL, Synthesis of adenosine triphosphate and exchange between inorganic phosphate and adenosine triphosphate in sodium and potassium ion transport adenosine triphosphatase. *J Biol Chem* **250:** 3010–3018, 1975.
25. Hara Y and Nakao M, Sodium ion discharge from pig kidney Na^+,K^+-ATPase. Na^+-dependency of the E1P $\rightleftharpoons$ E2P equilibrium in the absence of KCl. *J Biochem (Tokyo)* **90:** 923–931, 1981.
26. Yoda A and Yoda S, Formation of ADP-sensitive phosphorylated intermediate in the electric eel Na,K-ATPase preparation. *Mol Pharmacol* **22:** 693–699, 1982.