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# Interaction of β-eudesmol with $Na^+,K^+$-ATPase: inhibition of $K^+$-pNPPase activity

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**Abstract**—The effect of β-eudesmol, one of the major components in So-jutsu (Atractylodis Lanceae Rhizoma), on $K^+$-dependent *p*-nitrophenyl phosphatase ($K^+$-pNPPase) activity was studied. It inhibited $K^+$-pNPPase activity with an $I_{50}$ value of $4.1 \times 10^{-4}$ M. The inhibition rate decreased as the $K^+$ concentration was increased, whereas greater inhibition was observed with high concentrations of either $Na^+$ or ATP. The $K_i$ values for $Na^+$ in the presence of 0, 0.1 and 1 mM ATP were 140, 260 and 310 mM, respectively, but with the addition of β-eudesmol, these values decreased to 90 mM regardless of the ATP concentration. This study on $K^+$-pNPPase activity supports the conclusion obtained from the study on $Na^+,K^+$-ATPase activity (Satoh K *et al.*, *Biochem Pharmacol* **44**: 373–378, 1992) that is, β-eudesmol interacts with enzyme in the $Na \cdot E_1$ form and inhibits the reaction step $Na \cdot E_1 \rightarrow Na \cdot E_1$-P. Furthermore, in the study of the effects of $K^+$ and β-eudesmol on $K^+$-pNPPase activity, it was confirmed that β-eudesmol prevents the conformational change of $Na \cdot E_1 \rightarrow K \cdot E_2$.

So-jutsu (Atractylodis Lanceae Rhizomas), an important group of Chinese traditional drugs [1], is used to normalize kidney, stomach and intestine function. A typical clinical use is for the treatment of water retention in the body. So-jutsu contains 5–9% sesquiterpenoides as the major components, e.g. β-eudesmol and hinesol. The variety of activities of So-jutsu are attributed to the sesquiterpenoides.

$Na^+,K^+$-ATPase plays a role in the active transport of $Na^+$ and $K^+$ across the cell membrane [2]. In the kidneys, $Na^+$ and water are absorbed in tubules, especially where $Na^+,K^+$-ATPase is abundantly localized. β-Eduesmol was found to be a relatively specific inhibitor of $Na^+,K^+$-ATPase [3]. It inhibits $Na^+,K^+$-ATPase activity with an $I_{50}$ value of $1.6 \times 10^{-4}$ M, but it weakly inhibits $Ca^{2+}$-ATPase and $H^+,K^+$-ATPase activities and has little effect on $Mg^{2+}$-ATPase, $H^+$-ATPase and acid and alkaline phosphatases. The inhibition of both $Na^+,K^+$-ATPase activity and the phosphorylation reaction of enzyme protein were prominent with high concentrations of $Na^+$. The inhibition rate was not dependent on the $K^+$ concentration. β-Eudesmol had no effect on ADP-sensitive or $K^+$-dependent dephosphorylation reactions. It was assumed to interact with the enzyme in the $Na \cdot E_1$ form and to inhibit the reaction step $Na \cdot E_1 \rightarrow Na \cdot E_1$-P [3]. The present communication deals with the effect of β-eudesmol on $K^+$-dependent *p*-nitrophenyl phosphatase ($K^+$-pNPPase*), reflecting a reaction in the $E_2$ state of $Na^+,K^+$-ATPase [4].

## *Materials and Methods*

$Na^+,K^+$-ATPase was prepared from horse kidney outer medulla [5]. The sodium dodecyl sulfate-treated [5] enzyme (which has an $Na^+,K^+$-ATPase activity of 28–50 μmol/mg protein/min) was used for the assay of $K^+$-pNPPase activity. $K^+$-pNPPase activity was determined in a mixture containing 20 mM *p*-nitrophenyl phosphate (pNPP), 15 mM KCl, 10 mM $MgCl_2$ and 0.1 M Tris–HCl buffer (pH 7.7) [5]. When β-eudesmol (98% pure), purchased from Wako Pure Chemical Industries (Osaka, Japan), was included in the assay mixture, 10 μL of its solution in ethanol–dimethyl sulfoxide (ethanol–DMSO, 8:2, v/v) was added to a total volume of 500 μL. Ethanol and DMSO in the β-eudesmol solution had no apparent effect on $K^+$-pNPPase activity.

*Abbreviations: $K^+$-pNPPase, $K^+$-dependent *p*-nitrophenyl phosphatase; DMSO, dimethyl sulfoxide; and pNPP, *p*-nitrophenyl phosphate.

## *Results and Discussion*

*Inhibition of $K^+$-pNPPase activity by β-eudesmol.* $K^+$-pNPPase activity was inhibited by β-eudesmol in a concentration-dependent manner and was inhibited almost completely at $10^{-2}$ M (Fig. 1). The apparent $I_{50}$ value of β-eudesmol for $K^+$-pNPPase activity was $4.1 \times 10^{-4}$ M. In a study with various concentrations of substrate, the mode of inhibition was found to be noncompetitive with respect to pNPP, as judged from a Lineweaver–Burk plot of the data. The $I_{50}$ values of ouabain for $K^+$-pNPPase activity, determined in the presence and absence of β-eudesmol, were about the same ($5.1 \times 10^{-6}$ M). This result indicates that β-eudesmol interacts with the enzyme at a site other than the ouabain binding site.

*Effect of β-eudesmol on the stimulation of $K^+$-pNPPase activity by $K^+$.* The $K^+$-pNPPase activity was determined at various concentrations of $K^+$. The inhibition of $K^+$-pNPPase activity by β-eudesmol ($4.1 \times 10^{-4}$ M) decreased, as the $K^+$ concentration was increased. The lowest inhibition

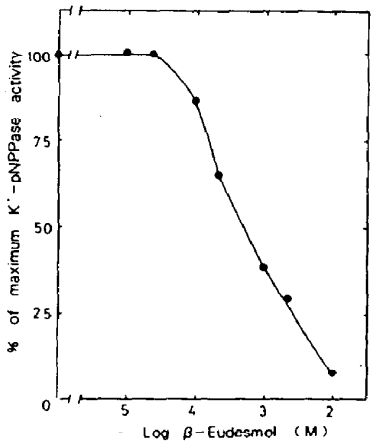


Fig. 1. Inhibition of $K^+$-pNPPase activity by β-eudesmol. Enzyme activity was determined with 0.5 μg of enzyme in the presence of various concentrations of β-eudesmol in ethanol–DMSO (8:2, v/v). The activity without β-eudesmol was taken as 100% (5.9 μmol/mg protein/min). The solvent for β-eudesmol was included in each control. The SD was less than 2.0%.

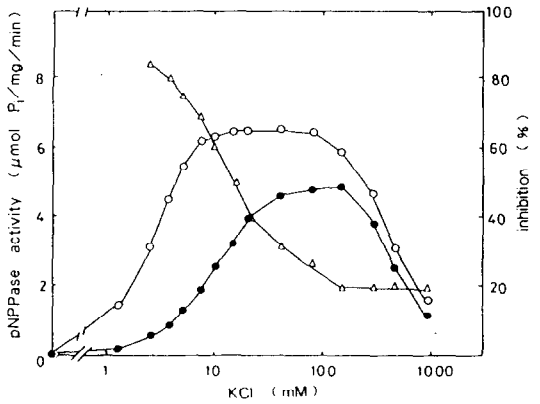


Fig. 2. Effect of β-eudesmol on $K^+$-pNPPase activity in the presence of various concentrations of $K^+$. Enzyme activity was determined with (●) or without (○) β-eudesmol ($4.1 \times 10^{-4}$ M). The inhibition rate at each $K^+$ concentration is shown (△). The SD was less than 2.0%.

rate, about 20%, remained unchanged at $K^+$ concentrations higher than 150 mM. Maximal activity attained at 150 mM $K^+$ in the presence of β-eudesmol was only 74% of that in the absence of β-eudesmol at 15 mM $K^+$. Thus, the inhibition of enzyme activity by β-eudesmol could not be restored completely by increasing the $K^+$ concentration (Fig. 2). $K_{0.5}$ values, obtained from Hill plots, for $K^+$ in the presence and absence of β-eudesmol were 9.5 and 2.4 mM, respectively. $K^+$-pNPPase activity reflects the enzyme conformation of the $K \cdot E_2$ form [4]. On the assumption that β-eudesmol interacts with the enzyme in the $Na \cdot E_1$ form [3], it may prevent the transition from $Na \cdot E_1$ to $K \cdot E_2$.

*Effect of β-eudesmol on $K^+$-pNPPase activity in the presence of $Na^+$ and/or ATP.* When the concentration of $Na^+$ was varied, the extent of inhibition by β-eudesmol remained at about 50%, being independent of the $Na^+$ concentration in the range up to 25 mM (Fig. 3). However, at $Na^+$ concentrations higher than 25 mM, the inhibition rate increased as the $Na^+$ concentration was increased. The $K_i$ values for $Na^+$ were 90 and 140 mM in the presence and absence of β-eudesmol, respectively (Fig. 4a). Similarly. the enzyme activity was examined in the presence of various

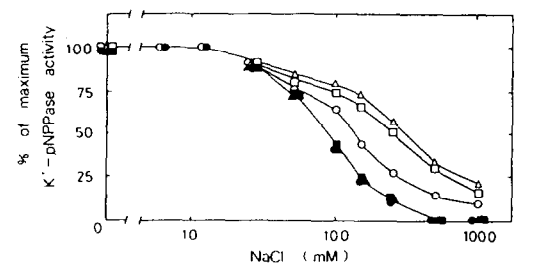


Fig. 3. Effect of β-eudesmol on $K^+$-pNPPase activity in the presence of various concentrations of $Na^+$ and/or ATP. Enzyme activity was determined with (closed symbols) or without (open symbols) β-eudesmol ($4.1 \times 10^{-4}$ M). Various concentrations of NaCl were included in the absence (○, ●) or presence of 0.1 (□, ■) or 1 mM (△, ▲) ATP. These enzyme activities without $Na^+$ at each condition were taken as 100%. They were 8.8, 4.4, 8.5, 4.1, 6.6 and 1.9 μmol/mg protein/min for ○, ●, □, ■, △ and ▲, respectively. The SD was less than 2.4%.

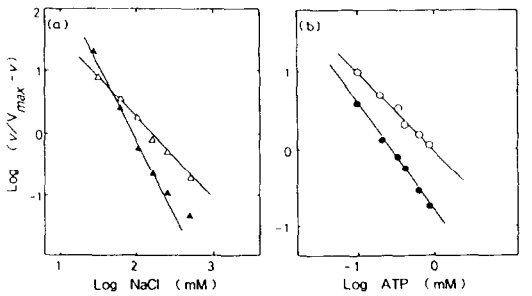


Fig. 4. Hill plots of $K^+$-pNPPase activity in the presence and absence of β-eudesmol with various concentrations of $Na^+$ or ATP. Enzyme activity was determined with (closed symbols) or without (open symbols) β-eudesmol ($4.1 \times 10^{-4}$ M). (a) NaCl or (b) ATP was included at various concentrations. The SD was less than 2.3%.

concentrations of ATP (Fig. 4b), and it was found that the inhibition by β-eudesmol was greater when the ATP concentration was higher. The $K_i$ values for ATP were 0.26 and 0.95 mM in the presence and absence of β-eudesmol, respectively (Fig. 4b). β-Eudesmol may act cooperatively with $Na^+$ or ATP, increasing the affinity of each ligand for the enzyme, in the inhibition of $K^+$-pNPPase activity.

The effect of β-eudesmol ($4.1 \times 10^{-4}$ M) on $K^+$-pNPPase was examined under conditions where ATP and $Na^+$ were present simultaneously (Fig. 3). The $K_i$ value for $Na^+$ was increased from 140 mM to 260 and 310 mM by the addition of 0.1 and 1 mM ATP, respectively (Fig. 3), whereas these values were decreased to 90 mM in the presence of β-eudesmol regardless of the concentration of ATP (Fig. 3). It is known that the inhibition of $K^+$-pNPPase activity by $Na^+$ is attenuated by the addition of ATP, accompanied with an increase of the $K_i$ value for $Na^+$ [6]. The underlying mechanism is that $Na^+$ favors transition of the enzyme to $Na \cdot E_1$ from $K \cdot E_2$, whereas ATP favors the overall reaction leading to the $E_2$ form via $Na \cdot E_1$-P [6]. In the presence of β-eudesmol, the inhibition by $Na^+$ was not reversed by the addition of ATP. These results support the idea that the reaction step from $Na \cdot E_1$ to $Na \cdot E_1$-P, which requires ATP, is blocked by β-eudesmol.

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