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A New Triterpenoid Saponin (Chromosaponin I) with a Reducing Power

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γ-Pyronyl-triterpenoid saponin (chromosaponin I; CSI) isolated from pea is strongly adsorbed at a glassy-carbon electrode, and gives an irreversible oxidation wave at 0.4 V *vs.* Ag/AgCl. The γ-pyronyl moiety is responsible for the electroactivity. CSI can reduce 1,4-benzoquinone, some of its derivatives and also cytochrome *c*. From comparison with other reductants, the formal redox potential of CSI was estimated to be 0.3–0.4 V *vs.* NHE (pH 7.0).

Recently a new type of triterpenoid saponin **1** was isolated from pea[1] and soybean[2] and found to be a conjugate of 3-hydroxy-2-methyl-4-pyrone (γ-Pyr) **2** and soyasaponin I (SI) **3** (Fig. 1). This compound showed an absorption maximum at 295 nm, and was named "chromosaponin I" (CSI) as a candidate for a UV-B photoreceptor.[1] Since it has been revealed that CSI is a naturally occurring form of SI (one of the major saponins in leguminous plants),[1] much attention is being paid to the physiological roles of CSI. In the present study, voltammetric and spectrophotometric measurements were performed to show that CSI possesses a definite reducing power. It should be noted that CSI is a unique *electroactive* saponin.

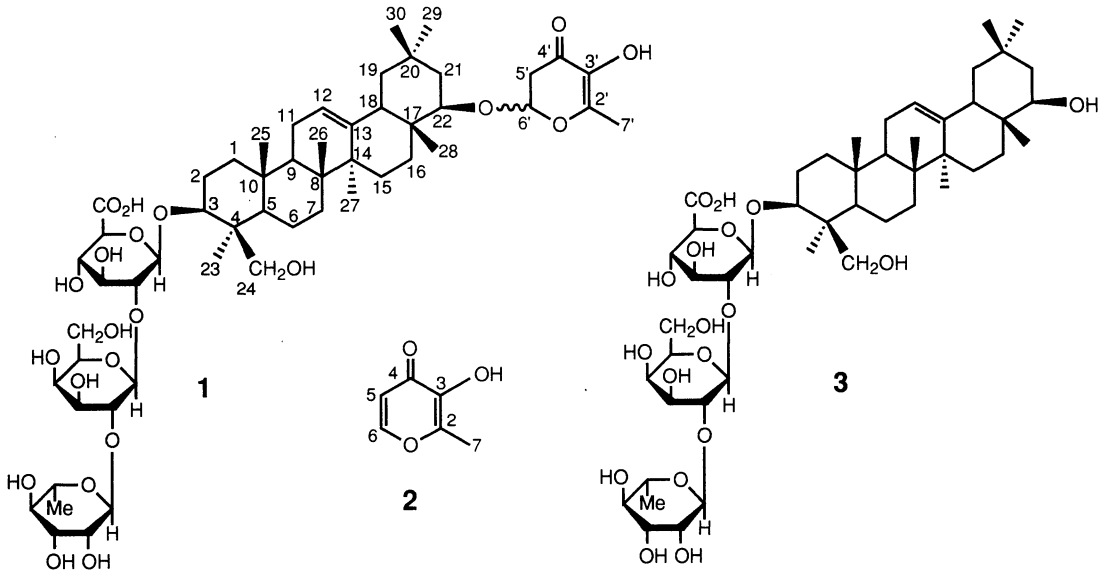

Fig. 1. Chemical structures of 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl(1→)]-22-*O*-[3'-hydroxy-2'-methyl-5',6'-dihydro-4'-pyrone(6'→)]- 3β, 22β, 24-trihydroxy-olean-12-ene (CSI) (**1**), 3-hydroxy-2-methyl-4-pyrone (γ-Pyr) (**2**), and soyasaponin I (SI) (**3**).

CSI used in this study was isolated from pea seedlings (*Pisum sativum* L. cv Alaska) according to a previous procedure[1] with slight modification (the details will be presented elsewhere). SI was prepared from CSI as described previously.[1] Voltammetric measurements were performed with a laboratory-constructed microcomputer-controlled system. A three-electrode system was employed with a glassy-carbon (GC) working electrode (Tokai Carbon, GC-30S; surface area = 0.071 cm^2), a platinum counter electrode, and an Ag/AgCl (saturated KCl) reference electrode. The GC electrode surface was polished with 0.06 μm alumina and 0.25 μm diamond slurry successively. Unless otherwise noted, the electrode surface was freshly polished with the diamond slurry for each record of a voltammogram and washed in an ultrasonic field with distilled water, and ethanol, and finally with distilled water. Test solutions were deaerated with N_2 gas. The electrolytic cell was water-jacketed to maintain the temperature at 25 °C. For spectrophotometric measurements, Jasco Model Ubest-30 spectrophotometer was used.

Figure 2(A) shows cyclic voltammograms obtained with a GC electrode in the presence of (a) 0.02, (b) 0.05, and (c) 0.2 mM CSI in 0.1 M phosphate buffer (pH 7.7) (M = mol dm^{-3}). In all cases, a well-developed anodic peak appeared around 0.45 V, though the peak potential was somewhat affected by the CSI concentration. It should be noted that the anodic peak was not followed by a cathodic peak on the reverse scan, indicating that CSI was decomposed immediately after the electrochemical oxidation. At voltage scan rates $v \leq 5.0$ V s^{-1}, such an irreversible wave was always observed.

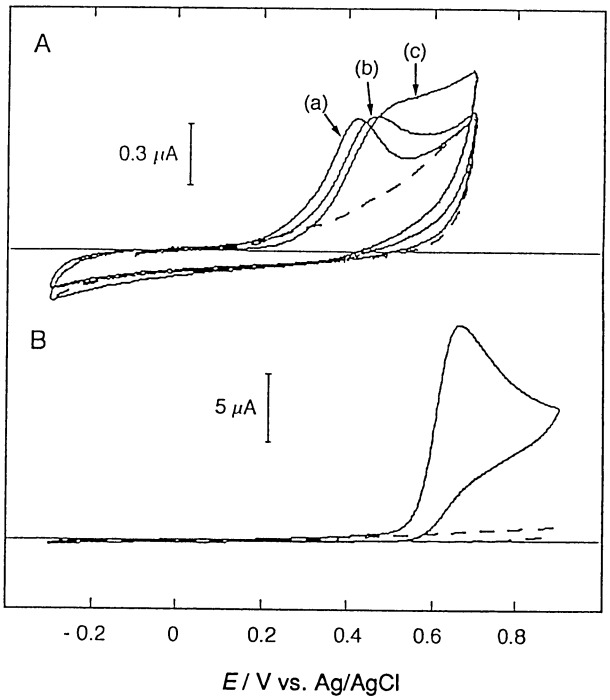

Fig. 2. Cyclic voltammograms of (A) CSI and (B) γ-Pyr recorded with a GC electrode in 0.1 M phosphate buffer (pH 7.7). (A) CSI concentrations: (a) 0.02, (b) 0.05, and (c) 0.2 mM. (B) γ-Pyr concentration: 1.0 mM. Broken lines are base currents. The voltage scan rate: 0.1 V s^{-1}.

As shown in Fig. 2(B), γ-Pyr also gave an irreversible anodic wave. The anodic peak potential of CSI was more negative by > 0.15 V than that of γ-Pyr, suggesting that the γ-pyronyl group was more susceptible to oxidation than its free form. In contrast to γ-Pyr, SI gave no redox wave. These results clearly indicate that the γ-pyronyl moiety serves as an electroactive site in CSI. The anodic peak current of γ-Pyr was proportional to the square root of v (0.02–0.4 V s^{-1}), indicating that the oxidation process was diffusion-controlled.

The cyclic voltammogram of CSI has characteristics of an adsorption wave.[3] The anodic peak current (I_{pa}) was not very dependent on the CSI concentration. As shown in Fig. 3, I_{pa} reached a constant only at 0.005 mM and then increased gradually at the concentrations > 0.2 mM. Also, I_{pa} was proportional to v (not to $v^{1/2}$ for a diffusion-controlled process) in the range of 0.02 to 0.4 V s^{-1}. These dependences may be elucidated in terms of the adsorption of CSI on the electrode surface. The adsorption process seems to be very rapid, because the peak currents were reproduced even if voltammograms were recorded only one minute after dipping the electrode in the test solution.

In order to examine the adsorption behavior of CSI on the electrode surface, the following experiments were performed: Onto a GC electrode surface, CSI was adsorbed by dipping the electrode for one minute in a phosphate buffer (pH 7.7) containing a lower concentration (0.04 mM) or a higher concentration (1.0 mM) of CSI. The CSI-adsorbed electrode prepared in this manner was then transferred into a deaerated buffer solution and allowed to stand for one minute with or without stirring (by bubbling N_2). In each case, a voltammogram was recorded after the solution became calm. Curve (a) in Fig. 4(A) and 4(B), being obtained without stirring, was almost identical to that recorded in a CSI-containing solution (curves (a) and (c) in Fig. 2(A)). As for the electrode prepared with a dilute CSI solution, the voltammograms were hardly influenced by stirring prior to the voltammetric measurements (curves (a) and (b) in Fig. 4(A)). These results showed that CSI was strongly (*i.e.* irreversibly) adsorbed on the electrode surface at the low concentration. On the other hand, the voltammogram obtained at the electrode prepared with a relatively concentrated CSI solution was highly affected by stirring (compare curves (a) and (b) in Fig. 4(B)). Since the area of the anodic peak became smaller in curve (b), a part of CSI, being adsorbed weakly, would be detached from the electrode surface by stirring. These results suggest that CSI is adsorbed at the electrode in two states; in one state, CSI exists in a monomolecular layer on the electrode surface, and in the other state, multimolecular layers are possibly formed.

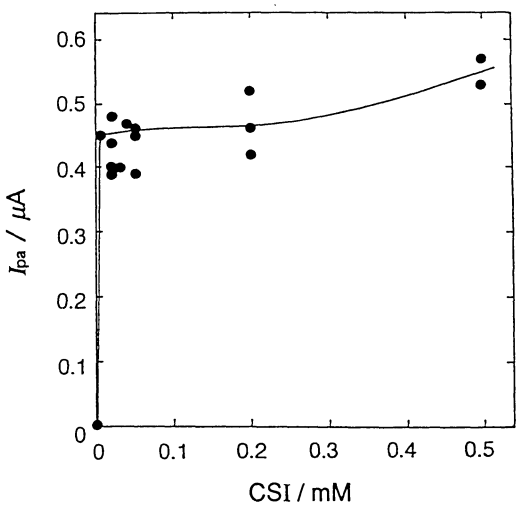

Fig. 3. Effect of the CSI concentration on the anodic peak current (I_{pa}). Experimental conditions as in Fig. 2.

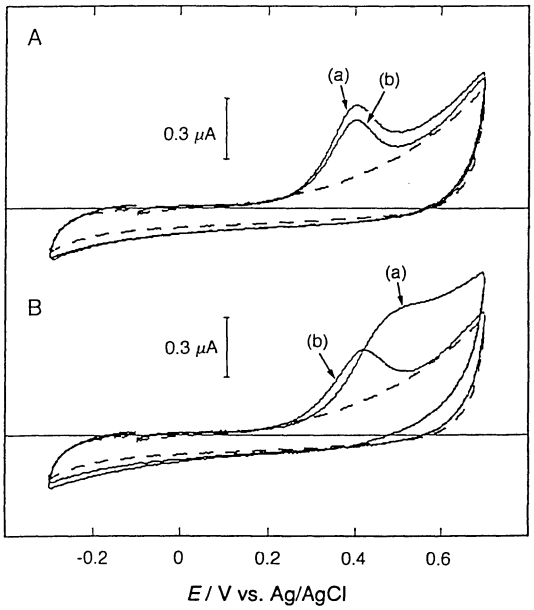

Fig. 4. Cyclic voltammograms showing the adsorption behavior of CSI on the GC electrode surface. See text for details.

The number of electrons required for the oxidation of CSI could not be determined unequivocally by controlled potential electrolysis because of an unceasing current presumably due to the successive oxidation of the oxidation product(s). However a kinetic analysis of the reduction of 1,4-benzoquinone (BQ) by CSI has clearly demonstrated that two electrons participate in the oxidation of CSI.[4)]

To estimate the reducing power of CSI, voltammetric measurements were extended to some biological reductants, *viz.* ascorbate, urate, and cysteine. All of these compounds gave irreversible waves similar to that observed for γ-Pyr, the anodic peak currents being proportional to $v^{1/2}$. The anodic peak potentials (E_{pa}) at 0.1 V s^{-1} were 0.35, 0.40, and 0.79 V *vs.* Ag/AgCl (pH 7.7) for ascorbate, urate, and cysteine, respectively (cf. E_{pa}

= 0.42–0.51 V for CSI at the same pH). Although a strict comparison of the E_{pa} values is not pertinent for different compounds giving irreversible waves, this result implies that CSI may possess a reducing power stronger than cysteine and comparable to ascorbate and urate at pH 7.7.

To make sure of its reducing power, CSI was allowed to react with BQ, and the reaction was monitored UV-spectrophotometrically. As we expected, BQ was reduced by CSI as shown by curve (B) in Fig. 5. In this figure, the reducing power of CSI against BQ is compared with those of some reductants including ascorbate, Trolox (2-carboxy-2,5,7,8-tetramethyl-6-chromanol, a water soluble analogue of vitamin E), and urate. The reducing power becomes stronger in the order: urate < Trolox < CSI < ascorbate (ascorbate$^{\bullet-}$, H^+/ascorbate monoanion). This order is in harmony with the formal redox potentials ($E^{\circ\prime}$) of urate, Trolox, and ascorbate, *i.e.,* 0.590, 0.480, and 0.282 V *vs.* NHE at pH 7.0, respectively.[5] Accordingly, the $E^{\circ\prime}$-value for CSI may be expected to lie between 0.3 and 0.4 V.

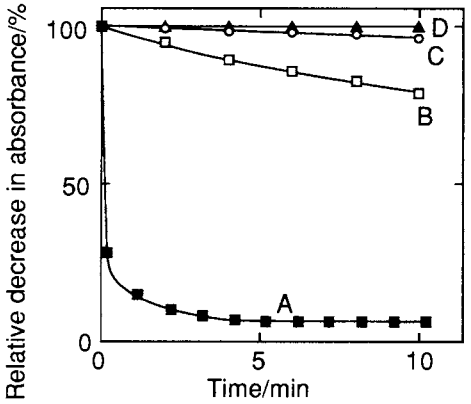

Fig. 5. Reductions of BQ by (A) ascorbate, (B) CSI, (C) Trolox, and (D) urate. The reaction cell contained 0.1 mM BQ, 0.1 mM reductant (ascorbate, CSI, Trolox, or urate), and 0.1 M phosphate buffer (pH 7.7). Absorbance changes were followed at 245 nm.

In addition, the reductions of some quinone derivatives, *i.e.,* methyl-1,4-benzoquinone (MBQ) and 2,5-dimethyl-1,4-benzoquinone (2,5-DMBQ), and cytochrome *c* (Cyt *c*) by CSI were examined. CSI could reduce MBQ ($E^{\circ\prime}$ = 0.22 V *vs.* NHE, pH 7.0) and Cyt *c* ($E^{\circ\prime}$ = 0.26 V),[5] but could not effectively reduce 2,5-DMBQ ($E^{\circ\prime}$ = 0.18 V).[6] Thus, CSI can reduce the compounds with $E^{\circ\prime} \geq$ about 0.2 V.

The present results suggest a possibility of the participation of CSI as an electron donor *in vivo.* Since CSI is surface-active as observed in the adsorption behavior at the GC electrode surface, it is reasonable to expect that CSI shows certain affinity to biomembranes.

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