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Reduction of Chromium(VI) by Water-Extracts from Withered Oak Leaves

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Introduction of water-extracts from withered oak leaves into potassium chromate readily diminished Cr(VI) to give water soluble chromium species which are presumably composed of tannin and Cr(III). Thus, this method is useful for treatment of potential hazards like Cr(VI).

The major valences of Cr naturally present are hexavalent (VI) and trivalent (III), and Cr(VI) is known to be more toxic than Cr(III) because of rapid invasion into the cells. Thus, the behavior of the Cr(VI) in the environment has to be carefully observed. In general, chromate ion or dichromate ion is not adsorbed to soils. As the result, Cr(VI) in the chemical disposal sites is transported to the environment, causing water pollutions. Under this circumstances, it is urgent to find some means to get rid of such a toxic material. Recent our trial introducing ground withered oak leaves into Cr(VI)-containing waters readily diminished Cr(VI), thus prompting us to investigate the mechanism. In the meanwhile many organic compounds have shown to reduce Cr(VI) to Cr(III). Recently, the reduction by saccharides has been shown to proceed by conversion of Cr(VI) into soluble Cr(III) species via Cr(V) intermediate species.1) We describe in this report that water-extract from withered oak leaves converts Cr(VI) to water soluble species presumably composed of tannin and Cr(III).

Withered leaves were collected from oak trees and ground by high speed grinder (Flitsch, Germany, 15-301). The resulting powder (0.5 g) was mixed with water (50 ml) and heated in a water bath at 100 °C for 1 h. The mixture was centrifuged at a low speed to obtain supernatant, which was used as a water-extract. A mixture of the water-extract (5 ml) and 2 mM (1 M=1 mol $\mathrm{dm^{-3}}$) potassium chromate solution (5 ml) was incubated at room temperature for various periods. The Cr(VI) in the mixture was spectrophotometrically determined using diphenylcarbohydrazide,2) and tannin by the method using ferrous tartrate and ethyl gallate as a standard.3)

Figure 1 shows the time courses of Cr(VI) and tannin concentrations in the reaction mixture. Almost all the Cr(VI) disappeared, and 60% of the total tannin disappeared at the same incubation period. This suggests that the Cr(VI) in the mixture is reduced with concomitant oxidation of the tannin. The amount of Cr(VI) disappeared was estimated to be 490 µg. In general, the reduction of Cr(VI) by organic compounds proceeds at acid condition. The pH of the reaction mixture just after preparation and incubation for 20 h were determined to be 5.50 and 6.90, respectively. This seems to be one of reasons that the reduction rapidly proceeds.

In the next step, the possible function of the tannin was investigated using Sephadex G-25 column (1 x 85 cm) equilibrated in water.4) Three reaction mixtures containing the water-extract (5 ml) and 2 mM potassium chromate solution (5 ml) were incubated at room temperature for 30 min, 2 h, and 18 h, respectively. 5 ml portion of each mixture was applied to the Sephadex G-25 column, followed by elution with water. Twenty fractions were collected, and tannin, Cr(VI), and total Cr in each fraction (fraction No. 6 to 15) were determined. The total Cr was determined by atomic absorption spectrometry (Hitachi, 180-30). Figure 2-(1) shows a chromatogram for a reaction mixture incubated for 30 min, Elution patterns of Cr and tannin were dissimilar to each other. The peak level of Cr(VI) was lower than that of total Cr. These

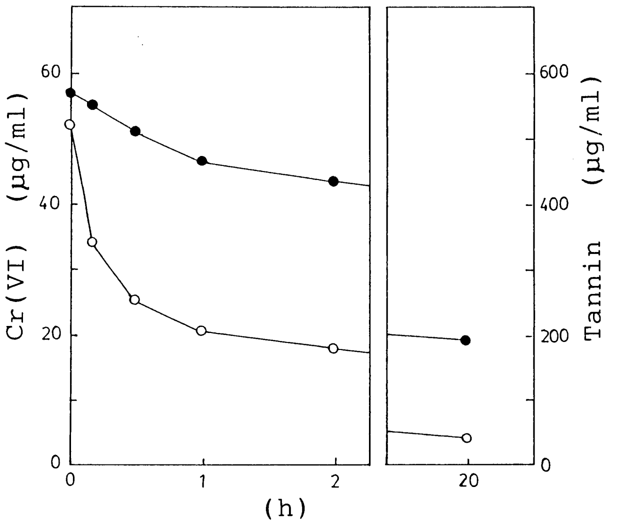

Fig. 1. Time courses of Cr(VI) (○) and tannin (●) concentrations in the mixture.

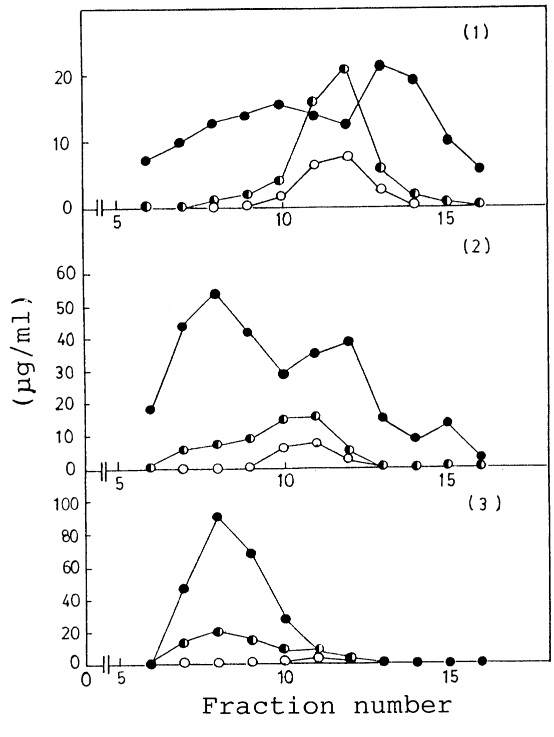

Fig. 2. Sephadex G-25 column chromatographies for mixtures incubated for 30 min (1), 2 h (2), and 18 h (3). ● , tannin; ◐ , total Cr; ○ , Cr(VI).

results suggest that reduction of Cr(VI) preferentially occurs at the beginning of the reaction. Figure 2-(2) shows a chromatogram for a reaction mixture incubated for 2 h. The elution patterns of total Cr and tannin overlap with each other, suggesting that interaction between tannin and Cr occurs. Figure 2-(3) shows a chromatogram after incubation for 18 h. Cr(VI) almost completely disappeared, and elution profiles of tannin and total Cr mostly overlap with each other. The amount of Cr is nearly consistent with that of Cr disappeared during incubation with the same condition. These results suggest that the reduced Cr should be composed to form a water soluble chromium species with the tannin. Chemical analysis of each fraction from the Sephadex G-25 column revealed that many fractions contain saccharides (data not shown). However, the elution patterns of saccharides and Cr was dissimilar to each other. This suggests that saccharides are not involved as components in the complex. Moreover, it is known that incubation of chromate ion with ascorbic acid in the presence of ethylenediaminetetraacetic acid (EDTA) formed Cr(III)-EDTA complex immediately after appearance of Cr(III) in the mixture.5) Similar reaction probably occurs in the present reaction between chromate ion and the water-extract. Further studies will be necessary to clarify the mechanism of the reaction.

The valence of the reduced Cr was then investigated by the modified procedure using trans-1,2-cyclohexadiamine-N,N,N',N'-tetraacetic acid (CyDTA).5) The reaction mixtures containing 2 ml of the water-extract and either 2 ml of potassium chromate solution (1 mM) or water (for control) in a total volume of 40 ml were incubated at room temperature for 1 h. Then 3 ml of 1 mM CyDTA was added to the mixtures, and the mixtures were adjusted to pH 3.0 with acetic acid followed by heating in a water bath at 100 °C for 15 min. After cooling down to room temperature, 57 ml of cold water, about 0.1 g of ascorbic acid, 4 ml of ammonia-ammonium chloride at pH 10.0 (containing 7 g of ammonium chloride and 57 ml of concentrated ammonium hydroxide in a total volume of 100 ml) and several drops of Eriochrome Black T indicater were added to the mixtures, and CyDTA left was titrated with 1 mM manganese(II) solution. This experiment was repeated three times. The average volume of manganese(II) ion to titrate CyDTA in the control and mixtures containing potassium chromate were calculated to be 2.90 ml and 0.96 ml, respectively. Thus, the amount of CyDTA consumed during the reaction is calculated to be 1.94 µmol, indicating that the same amount of Cr(III) was produced. Finally, almost all the Cr (2 µmol) added was most probably converted to the complex with CyDTA through Cr(III)-tannin.

We then carried out characterization for the tannin in the water-extracts. The water-extracts from withered oak leaves were hydrolyzed in 5% sulfuric acid at 100 °C for 6 h,$^{6)}$ and the hydrolysates were extracted with ethyl acetate. The ethyl acetate-extract was condensed by rotary evaporator. The hydrolysates in the condensed extract, the nontreated water-extract, and gallic acid were subjected to paper chromatography in a solvent system of 1-butanol : acetic acid : water (4:1:5, V/V/V upper phase).$^{7)}$ Phenolic compounds on the chromatogram were analyzed by dipping in ferrous chloride-potassium ferricyanide solution.$^{8)}$ The hydrolysates gave four major blue spots whose mobilities relative to gallic acid [R(gallic acid)] are 0.00, 0.71, 0.98, and 1.21, respectively. Consequently, the compound with R(gallic acid)=0.98 is most likely gallic acid. The nontreated water-extract gave only one blue spot remaining at the origin of the chromatogram. It is known that oak leaves have two types of tannins, one is hydrolyzable tannin involving gallic acid, the other is condensed tannin involving catechin.$^{9)}$ Thus, the water-extract prepared from withered oak leaves most probably contains hydrolyzable tannin. Taken together, the water-extract seems to convert Cr(VI) to Cr(III) to form a water soluble chromium species with tannin. Therefore, the introduction of the water-extract from withered leaves or withered leaves themselves is a useful means for practical scavenging of ecological hazards like hexavalent Cr.

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