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Relationship Between Tyrosinase Inhibitory Action and Oxidation-Reduction Potential of Cosmetic Whitening Ingredients and Phenol Derivatives

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The oxidation-reduction potentials of cosmetic raw materials, showing tyrosinase inhibitory action, and phenolic compounds structurally similar to L-tyrosine were determined by cyclic voltammetry. The voltammograms obtained could be classified into 4 patterns (patterns 1-4). Pattern 1, characterized by oxidation and reduction peaks as a pair, was observed with catechol, hydroquinone or phenol, and pattern 2 exhibiting another oxidation peak in addition to oxidation and reduction peaks as a pair was found with arbutin, kojic acid, resorcinol, methyl p-hydroxybenzoate and L-tyrosine as the substrate of tyrosinase. Pattern 3 with an independent oxidation peak only was expressed by L-ascorbic acid, and pattern 4 with a reduction peak only at high potentials, by hinokitiol. The tyrosinase inhibitory activity of these compounds was also evaluated using the 50% inhibitory concentration (IC_{50}) and the inhibition constant (Ki) as parameters. Hinokitiol, classified as pattern 4, showed the highest inhibitory activity (lowest IC_{50} and Ki). Hydroquinone showing the second highest activity belonged to pattern 1, which also included compounds showing no inhibition of tyrosinase activity. The inhibitory activity of compounds exhibiting pattern 2 was relatively low with Ki values being in the order of 10^{-4} M. Although there was no consistent relationship between oxidation-reduction potentials and tyrosinase inhibitory action, the voltammetry data can be used as an additional index to establish the relationship between the structure and the tyrosine inhibitory activity.

Key words : Phenol derivative, Cosmetic whitening ingredient, Tyrosinase inhibitory action, Oxidation-reduction potential, Cyclic voltammetry

INTRODUCTION

Melanin is one of the important factors in the determination of skin color. Epidermal pigmentations due to sunburn, chloasma, freckles, and senile pigment maculate are considered to be caused by an imbalance between melanin synthesis and melanin excretion, resulting in an abnormal increase in melanin in the epidermis (Morioka and Yamaguchi, 1983). These diseases have been treated with phenol derivatives such as hydroquinone and resorcinol that inhibit a series of reactions in the conversion of from L-tyrosine to melanin by inhibiting the tyrosinase activity (Takano, 1984). In addition, many cosmetics for whitening the skin contain L-ascorbic acid and its derivatives, kojic acid, arbutin, and so on, and they target the prevention of pigmentation. Since L-dopa and dopachrome are produced as oxidation intermediates in the enzymatic conversion of L-tyrosine to melanin, the oxidation-reduction potential of the melanin biosynthesis inhibitors was supposed to be related to their inhibitory action. In the present study, we measured the oxidation-reduction potential of cosmetic whitening ingredients and phenol derivatives by cyclic voltammetry. We also assessed the whitening effect of these compounds by determining their potency in the inhibition of tyrosinase specific for an oxidation reaction of L-tyrosine as an index (Pomerantz and Li,1970; Prota et al.,1981). Finally, we discussed the relationship between tyrosinase inhibitory action and reduction potential of the cosmetic whitening ingredients and phenol derivatives.

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MATERIALS AND METHODS

Cosmetic raw materials and chemicals

Cosmetic raw materials used were of the same grade described in the Japan Cosmetic Ingredients Dictionary, 4th ed., (Japan Cosmetic Industry Association, 1997). Phenol derivatives and other chemicals were reagent grade. Tyrosinase from mushroom was obtained from Sigma Co. (St. Louis, MO, USA). The chemical structures of some compounds used in this experiment are listed in Chart 1.

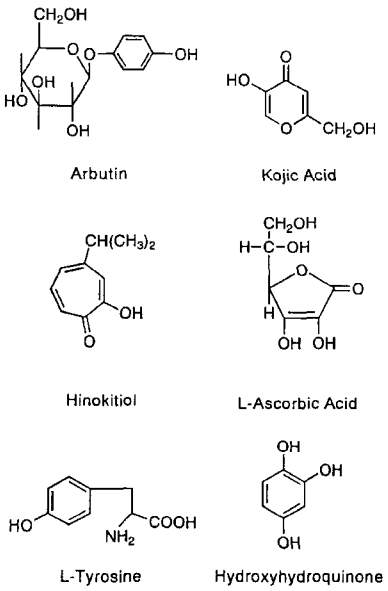

Chart. 1. Chemical structures of some compounds used in this experiment

Determination of protein

The amount of protein was measured as according to Lowry's method (Lowry et al., 1951) using bovine serum albumin as a standard.

Measurement by cyclic voltammetry

Cyclic voltammetry was performed using an electrochemical analyzer (model 100B, BAS), a GCE glassy carbon (BAS) as a working electrode, a platinum electrode as a counter electrode, and a silver-silver chloride electrode (RE 1B, BAS) as a reference electrode (Takamura et al., 1986; Sharma and Sharma,1987). The sample(1 mM) was dissolved in 1/15 M Sörensen phosphate buffer (pH 6.8). The sample solution was placed in a glass cell for measurement, aerated with nitrogen for 10 min to eliminate dissolved oxygen, and light-shielded. Cyclic voltammetry was performed at a sweep rate of 100 mV/sec by one scanning of the range from +1,800 mV to -1,800 mV.

Measurement of tyrosinase inhibitory activity

The tyrosinase inhibitory activity of cosmetic whitening ingredients and phenol derivatives was determined by modifying the original methods of Pomerantz and Li (1970) and Prota et al. (1981). Each inhibitor solution (2 ml) was diluted with pH 6.8 phosphate buffer to a designated concentration. To the diluted sample was added 0.5 ml of L-tyrosine solution (0.5 mg/ml), 2.0 ml of 1/15 M Sörensen phosphate buffer (pH 6.8), and 0.5

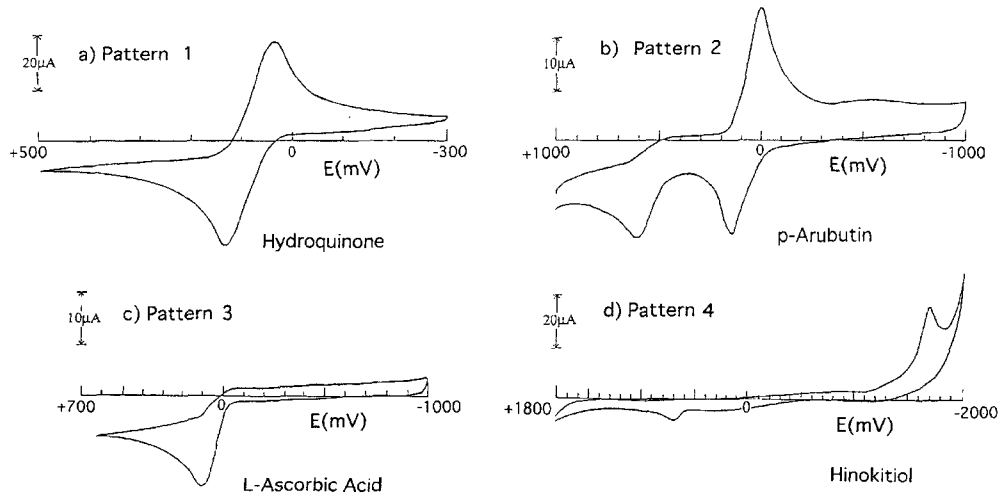

Fig. 1. Cyclic voltammetry of cosmetic whitening ingredients and phenol derivatives. The sample was dissolved in 1/15 M Sörensen phosphate buffer (pH 6.8) to obtain a concentration of 1 mM.The sample solution was placed in a glass cell for measurement, aerated with nitrogen for 10 min to eliminate dissolved oxygen, and light-shielded. Cyclic voltammetry was performed at a sweep rate of 100 mV/s by one scanning of the range from +1,800 mV to -1,800 mV.

Table I. Half-wave Potentials in Cyclic Voltammetry, 50% Inhibitory Concentration of Tyrosinase and Inhibition Constant of Cosmetic Whitening Ingredients and Phenol Derivatives

Pattern	Sample name	E1/2(mV)[a]	IC_{50} (M)[b]	Ki (M)[c]
1	Catechol	197		
	Hydroquinone[d]	85	5.1510^{-5}	4.7210^{-6}
	Phenol	17		
	Pyrogallol	117		
	Hydroxyhydroquinone[d]	117	2.3710^{-4}	
2	Arbutin[d]	73	7.4810^{-3}	3.7210^{-4}
	Kojic acid[d]	75	2.8510^{-4}	5.2410^{-4}
	L-Tyrosine	3		
	Resorcinol[d]	-20	1.4510^{-4}	1.4810^{-4}
	Methyl p-hydroxybenzoate[d]	5	1.5110^{-3}	3.2810^{-4}
	p-Nitrophenol[d]	-373	9.5310^{-4}	
	p-Fluorophenol	-17		
	p-Aminophenol	108		
	p-Methoxyphenol	64		
3	L-Ascorbic acid[d]		6.4010^{-4}	
	Phloroglucinol[d]		6.9410^{-4}	
4	Hinokitiol[d]		8.2210^{-6}	3.5610^{-7}

a) Half-wave potential
b) 50% inhibitory concentration
c) Inhibition constant
d) These samples had tyrosinase inhibitory.

ml of tyrosinase solution (153 g/ml) in 1/15 M Sörensen phosphate buffer (pH 6.8) at 37±0.1°C, and the absorbance (S) was measured at 475 nm. To measure enzyme activity in the absence of the inhibitor, 2.0 ml of 1/15 M Sörensen phosphate buffer (pH 6.8) was added instead of the sample. Absorbance (B) after the reaction was measured at 475 nm. Additionally, to determine the effects of coloring of the sample solution, 5 ml of 1/15 M Sörensen phosphate buffer (pH 6.8) was added instead of the tyrosinase solution, and absorbance (C) after the reaction was measured at 475nm. The inhibitory activity (%) was calculated using the following equation.

$$\text{Inhibitory activity (\%)} = \{B-(S-C)\}/B \times 100$$

Determination of Inhibitory constant (Ki)

L-Tyrosine solution (0.5 ml), at a concentration of 0.10, 0.25 or 0.50 mg/ml, was mixed with 0.5 ml tyrosinase (0.1 mg/ml) in 1/15M Sörensen phosphate buffer (pH 6.8) and 1 ml of inhibitor solution in phosphate buffer (pH 6.8), and the mixture was incubated for 5 min at 37±0.1°C. Absorbance at 475 nm of the resulting solution(S) was measured. The 50% inhibitory concentration (IC_{50}) was used as the concentration of inhibitors to express 50 % inhibition of tyrosinase activity. The inhibitory constant (Ki) was determined from a Lineweaver-Burk plot.

RESULTS

Cyclic voltammetry

Cyclic voltammograms were classified into 4 typical patterns, and voltammograms of a representative compound showing each pattern are shown in Fig. 1. Fig. 1a shows a voltammogram of hydroquinone as a representative of pattern 1. In this pattern, reduction and oxidation peaks were paired during cathode scanning and anode scanning, respectively, and their difference

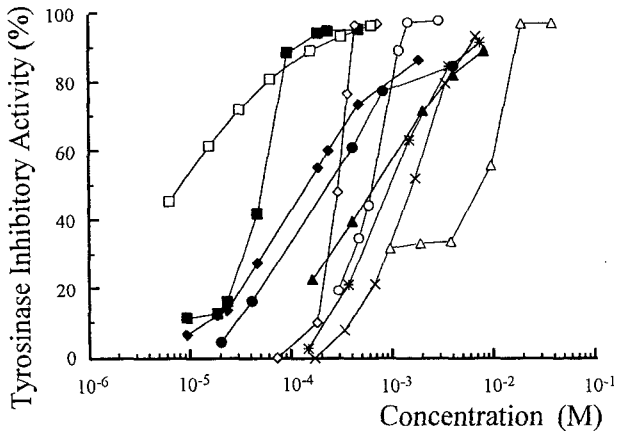

Fig. 2. Tyrosinase inhibitory activity of cosmetic whitening ingredients and phenol derivatives.□:Hinokitiol, ◇: Kojic Acid, ○:L-Ascorbic Acid, △:Arbutin, ■:Hydroquinone, ◆: Resorcinol, ● : Hydroxyhydroquinone, ▲ : Phloroglucinol, ×: Methyl p-hydroxybenzoate, ×: p-Nitrophenol Tyrosinase acti-vity was measured using L-tyrosine (8.22×10^{-6} M) as substrate. Results are expressed as a percentage of inhibition with respect to the untreated control. Values are the mean±S.D.

in potential was small. Fig. 1b demonstrates pattern 2 represented by arbutin. In this pattern, reduction and oxidation peaks also occurred as a pair during cathode scanning and anode scanning, but a second oxidation peak also occurred during anode scanning. The compounds showing this pattern included all the para-derivatives of phenol (Table I). The reduction peaks of all these compounds were observed between 45 and 320 mV, except p-nitrophenol which showed the reduction peak at -900 mV and no corresponding oxidation peaks. Fig. 1c shows a voltammogram of L-ascorbic acid (pattern 3). In this pattern, no reduction peak occurred during cathode scanning, and only an oxidation peak was observed during anode scanning. Pattern 4 was only observed for hinokitiol, as shown in Fig. 1d. A reduction peak occurred at -1,690 mV during cathode scanning. Table I also indicates the half-wave potential of each compound. This value can generally be utilized to evaluate the reversibility of reduction and oxidation reactions.

Tyrosinase inhibitory action of cosmetic whitening ingredients and phenol derivatives

Fig. 2 shows the tyrosinase inhibitory action of cosmetic whitening ingredients (L-ascorbic acid, hinokitiol, arbutin and kojic acid) and phenol derivatives (resorcinol, hydroquinone, phloroglucinol, hydroxyhydroquinone, p-nitrophenol, methyl p-hydroxybenzoate), expressed as the inhibitory activity (%) of each sample at each concentration. Their 50% inhibitory concentrations (IC_{50}) are shown in Table I. Among compounds tested, the most potent inhibition (IC_{50}, 8.22×10^{-6} M) was exhibited by hinokitiol, followed by hydroquinone, resorcinol, hydroxyhydroquinone, kojic acid, L-ascorbic acid, phloroglucinol, p-nitrophenol, methyl p-hydroxybenzoate and arbutin. The tyrosinase inhibitory action of these compounds was dose-dependent. In contrast, no tyrosinase inhibitory action was induced by phenol, catechol, pyrogallol, p-aminophenol, p-fluorophenol or p-methoxyphenol (data not shown).

Mode of inhibition by tyrosinase inhibitors

To evaluate the mode of inhibition by compounds with tyrosinase inhibitory activity and calculate their inhibitory constant (Ki), the enzyme inhibition was analyzed by Lineweaver-Burk plots. Fig. 3 demonstrates the Lineweaver-Burk plot of hinokitiol as a representative type of competitive inhibitor. Also, the same pattern of inhibition was observed with hydroquinone, resorcinol, methyl p-hydroxybenzoate and arbutin, and Ki values are shown in Table I. Meanwhile, the mode of inhibition by L-ascorbic acid (Fig. 4) was neither competitive nor non-competitive.

DISCUSSION

Cyclic voltammetry was performed (Takamura et. al., 1986; Sharma and Sharma, 1987) to evaluate the tyrosinase inhibitory action of phenol derivatives in terms of their oxidation-reduction potentials. Since methyl p-hydroxybenzoate, used as a preservative in cosmetics, and arbutin, a cosmetic whitening ingredient, are phenol derivatives, cosmetic raw compounds were examined for the oxidation-reduction potentials. Cyclic voltammo-

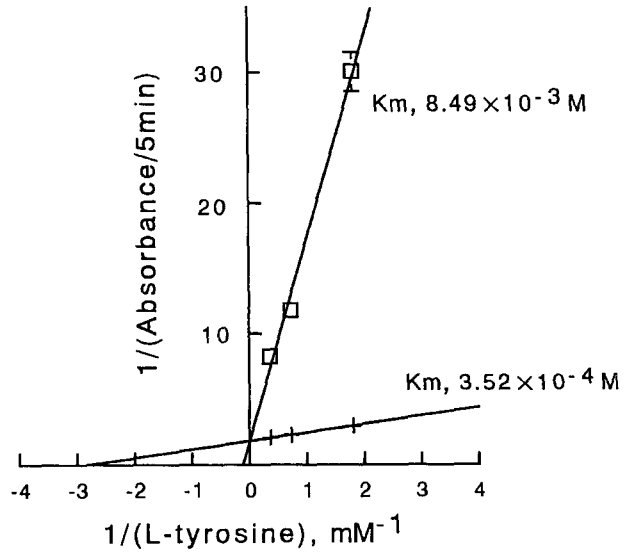

Fig. 3. Lineweaver-Burk plots of tyrosinase reaction and its inhibition by hinokitiol.This result shows the rate of the tyrosinase reaction in the presence (□) and absence (+) of hinokitiol (8.22×10^{-6} M). From this experiment, the inhibitor constant (Ki) for hinokitiol was determined using L-tyrosine as substrate. Values are the mean±S.D.

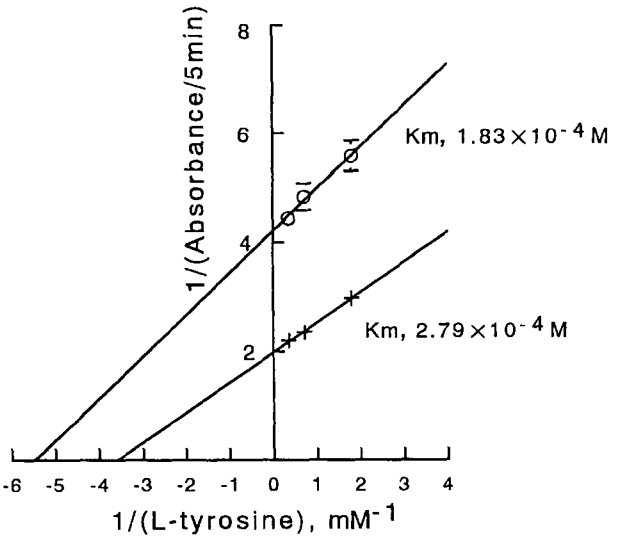

Fig. 4. Lineweaver-Burk plots of tyrosinase reaction and its inhibition by L-ascorbic acid.This result shows the rate of the tyrosinase reaction in the presence 6.40×10^{-4}M (○) and absence (+) of L-ascorbic acid. Values are the mean±S.D.

grams were classified into 4 patterns. Pattern 1 showed a small difference in the potential between the reduction and oxidation peaks (small half-wave potential), supporting a reversibility of reduction and oxidation reactions. This pattern was observed only with phenol derivatives bearing additional hydroxyl group. Pattern 2 was characterized by a second oxidation peak in addition to the reduction-oxidation peak pair. Pattern 3 was characterized by the absence of the peak pair and presence of only an independent oxidation peak. Both patterns 2 and 3 had an independent oxidation peak, which was also observed when anode scanning was not performed at high voltage, suggesting that oxidation reactions readily occurred. Only hinokitiol was categorized as pattern 4; a reduction peak was observed in the high voltage area during cathode scanning, and the reduced form was unstable. Next, we evaluated the tyrosinase inhibitory action of cosmetic whitening ingredients (Jpn. Cos. Ind. Assoc., 1997; Maeda and Fukuda, 1996) and phenol derivatives (Takano, 1984) that are used as melanin synthesis inhibitors. We found that all of the cosmetic whitening ingredients and some of the phenol derivatives, inhibitors of melanin biosynthesis, showed a moderate to high tyrosinase inhibitory action.

The relationship between chemical structure and tyrosinase inhibitory action led us to assume that the tyrosinase inhibitory action of the phenol derivatives containing 2 or 3 hydroxyl groups as substituents might be due to their role as reducing agents; resorcinol and phloroglucinol, with a hydroxyl group at the metaposition, and hydroquinone and hydroxyhydroquinone, with a hydroxyl group at the para-position, were sup-posed to inhibit tyrosinase. On the other hand, catechol and pyrogallol, with a hydroxyl group at the ortho-position, were thought to be stable and difficult to oxidize because of the formation of an inner chelate complex. This might explain the low inhibition of tyrosinase by those phenolic compounds.

Concerning the effect of substituent of para-substituted phenols, compounds with an amino group or a methoxy group as electron-donating groups showed no tyrosinase inhibitory activity, while those with a methoxycarbonyl group or a nitro group, electron-attracting groups, had a high tyrosinase inhibitory activity. These findings suggest that compounds with electron-attracting groups generally exhibited tyrosinase inhibitory activity through their reducing ability. However, p-fluorophenol having an electronegative substituent, was not consistent with this suggestion.

Finally, the relationship between cyclic voltammograms and tyrosinase inhibitory activity was examined by comparing IC_{50} and Ki, parameters of tyrosinase in-hibitory activity. Assuming that the tyrosinase inhibitory activity is due to the reducing role of the compounds, a special attention was paid to the oxidation peaks on cyclic voltammograms. Cyclic voltammograms could be classified into 4 patterns. Hinokitiol showing the highest inhibitory activity was classified as pattern 4, and hydroquinone showing the next highest activity as pattern 1. However, pattern 1 also included compounds without tyrosinase inhibitory activity. Compounds with tyrosinase inhibitory activity were frequently observed in pattern 2, although their activity was generally low (Ki value, about 10^{-4} M). The lack of a complete consistency between results of voltammetry and tyrosinase inhibitory activity may be ascribed to the different affinity in the association of phenolic compounds with tyrosinase.Therefore, results of voltammetry can not be used for direct estimation of enzyme inhibition, but can be used as an index for the relationship between the structure and enzyme inhibitory activity.

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