韓國營養學會誌 : 第17卷 · 第1號 · 1984
Korean J. Nutr. Vol. 17, No. 1, 1984

Inhibition Patterns of Dopachrome Formation as Influenced by Sulfur Dioxide

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SO_2에 의한 dopachrome 형성 억제 패턴

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=ABSTRACT=

Inhibiting action of sulfur dioxide (SO_2) on enzymatic discoloration was investigated with crude enzyme preparations from homogenized tissues or sliced disks of raw potato tubers. SO_2 appeared to inhibit the formation of dopachrome in a competitive manner. At insufficient concentrations of SO_2, the formation of dopachrome was reinitiated as time elapsed.

The present results suggested that SO_2 would form an additional compounds with certain intermediate during the course of enzymatic browning and delay the enzymatic discoloration. To inhibit the production of dopachrome in sliced disks of raw potato tubers, much higher concentrations of SO_2 were required than homogenized tissues.

INTRODUCTION

Dopachrome is the initially visible pigment concerned with the enzymatic browning of raw potato tubers. The discoloration pattern involving the formation and inhibition of dopachrome has been previously investigated in the presence of crude enzyme preparation from homogenized tissues and sliced disks of raw potatoes[1]. The results indicated that a much higher concentration of sulfur dioxide (SO_2) was required to inhibit the dopachrome production in sliced disks of raw potatoes than to bleach dopachrome which had been already formed.

Sulfur dioxide has been used as the inhibitor of enzymatic and nonenzymatic browning reactions for various foods[2] ; however, the inhibiting mechanism of SO_2 is not completely understood.

Enzyme – catalyzed oxidation of SO_2[3] and inactivation of potato polyphenol oxidase[4]

접수일자 : 1984. 1. 6.

have been reported in connection with the inhibiting mechanism of SO_2. According to Embs and Markakis[5], sulfite prevented browning by combining with the enzymatically produced o-quinone and stopping its condensation to melanin. Recently Muneta[6] reported that bisulfite inhibited blackening primarily by inhibiting the oxidation of tyrosine by polyphenol oxidase and also caused enzyme inactivation.

Since the degree of discoloration would be proportional to the dopachrome formed[7], the present study was undertaken to examine the inhibiting mechanism of SO_2 on the formation of dopachrome in the presence of crude enzyme preparation from homogenized tissues and sliced disks of raw potatoes. Various forms of sulfite are particularly applicable as sources of SO_2 since they are readily handled and prepared. Thus an attempt was made to determine the inhibiting efficiency of acid sulfite.

MATERIALS AND METHODS

'Irish Cobbler' potato was employed throughout the experiment. Crude enzyme preparation from homogenized tissues was based on procedures established by Muneta[7]. Patato disks 2 mm thick and 2cm in diameter were also used as enzyme source. Potato slices were cut in uniform thickness with a small rotary-type slicer and then disks were punched using a cork borer from storage tissue inside the vascular ring of prepared slices. The cortex portion was excluded since it is known to have high phenolic content[8]. Potato disks were immediately washed with running tap water for 10 minutes and blotted with filter paper.

The reaction mixture contained 0.5–3ml of 16mM 3, 4-dihydroxyphenylalanine(DOPA), 2ml of 0.2M phosphate buffer(pH 6), 1ml of crude enzyme preparation from homogenized tissues and 2ml of SO_2 solution. Various concentrations of SO_2 solution were prepared from $NaHSO_3$. Total volume was made up to 8ml with distilled water. It was allowed to react for 10 minutes. In the case of sliced disks, 5 disks were immersed in the above reaction mixture instead of adding crude enzyme preparation. It was allowed to react for 30 minutes. These experiments were proceeded at room temperature. The initial pH of the SO_2 solution was adjusted to 6 with 1M NaOH. All SO_2 concentrations involved in these experiments were based on stoichiometric values and data are presented as the final concentration of the reaction mixture.

The optical density of the reaction mixture was measured at 480nm with a Hitachi model 101 spectrophotometer(Hitachi Ltd., Tokyo, Japan). The reaction mixture containing no DOPA was used as the blank.

RESULTS AND DISCUSSION

It has been recognized that SO_2 is an effective inhibitor of enzymatic discoloration[3)9)10)]. In the presence of crude enzyme preparation or potato disks, the patterns of dopachrome formation closely resembled the general kinetic properties of enzymes[1]. Accordingly, the inhibiting mechanism of SO_2 on the formation of dopachrome was approached in a similar manner to studies of enzyme kinetics.

Fig. 1 shows a Lineweaver-Burk plot of inhibition of dopachrome formation by SO_2 in

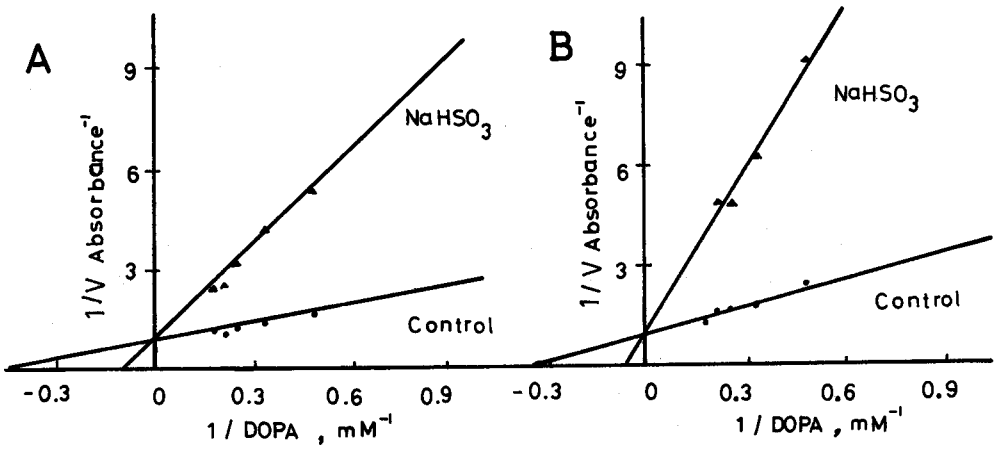

Fig. 1. Lineweaver–Burk plot of inhibition of dopachrome formation by SO_2 at enzyme preparation from homogenized tissues (A), or sliced potato disks (B). The pH of the SO_2 solution was 6.0. The stoichiometric SO_2 concentration was 20 μg/ml. Points are means of four replications.

the presence of a constant concentration of crude enzyme preparation(A) or a constant number of sliced disks(B). SO_2 apparently inhibited the formation of dopachrome in a competitive manner.

The Lineweaver–Burk plot of inhibition of dopachrome formation by various concentrations of SO_2 prepared from $NaHSO_3$ is shown in Fig. 2. The inhibiting patterns of dopachrome formation in the presence of crude enzyme preparation(A) and sliced potato disks(B)

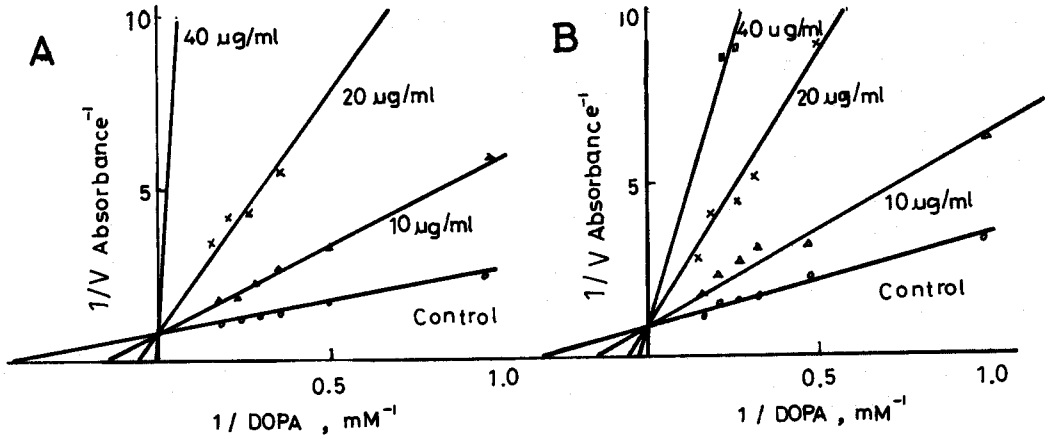

Fig. 2. Lineweaver–Burk plot of inhibition of dopachrome formation by various concentrations of SO_2 at constant concentration of enzyme preparation from homogenized tissues (A), or sliced potato disks (B). The pH of the SO_2 solution was 6.0. Points are means of four replications.

Table 1. The inhibiting effect of dopachrome formation by various concentrations of SO_2 in the presence of enzyme preparation from homogenized tissues and sliced potato disks[a].

Stoichiometric SO_2 concn (μg/ml)	Extent of inhibition (% of control)	
	Homogenized tissues	Disks
10	33.0	33.8
20	69.2	52.7
40	98.1	79.7

a. Initial pH of SO_2 solution was 6.0 and final concentration of DOPA was 6 mM.

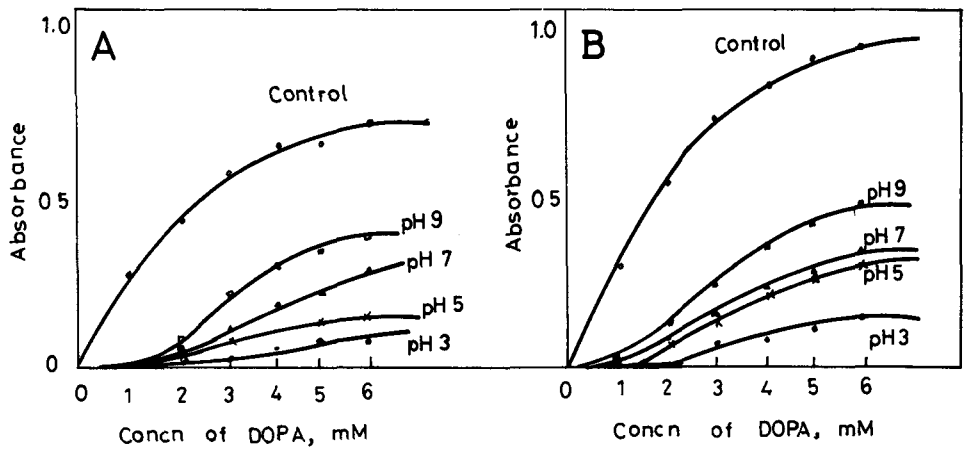

Fig. 3. The pattern of inhibition of dopachrome formation by various pH's of SO_2 in the presence of enzyme preparation from homogenized tissues(A), or sliced potato disks (B). The stoichiometric SO_2 concentration was 20 μg/ml. Points are means of four replications.

clearly indicate all concentrations of SO_2 act as competitive inhibitors. It has been recognized that competitive inhibitors are generally structural analogues of the substrate and compete with the enzyme at its active site[11]. Since SO_2 is not a structural analogue of the phenolic compounds used as substrate, the mechanism of competitive inhibition should be further elucidated.

The extent of inhibition of dopachrome formation by SO_2 was calculated as % of control in Table 1. The dopachrome formation was 30% inhibited by 10 μg/ml of SO_2, 50–70 % by 20 μg/ml of SO_2 and 80–100% by 40 μg/ml of SO_2. The degree of inhibition tended to be somewhat greater in the presence of crude enzyme preparation than sliced potato

disks. This might be related to the continuous production of the enzyme concerned with browning reactions in the presence of intact disk tissues. Less intimate contact of the SO_2 with the cells of the intact tissues might be also involved. Consequently, much higher concentration of SO_2 was required for inhibiting the production of dopachrome in the presence of potato disks than crude enzyme preparation.

According to Fig. 3, SO_2 tended to inhibit the dopachrome formation more the lower the initial pH of the SO_2 solution. The degree of inhibition was gradually reduced as the initial pH of the SO_2 solution increased to 9. Inhibition patterns were very similar

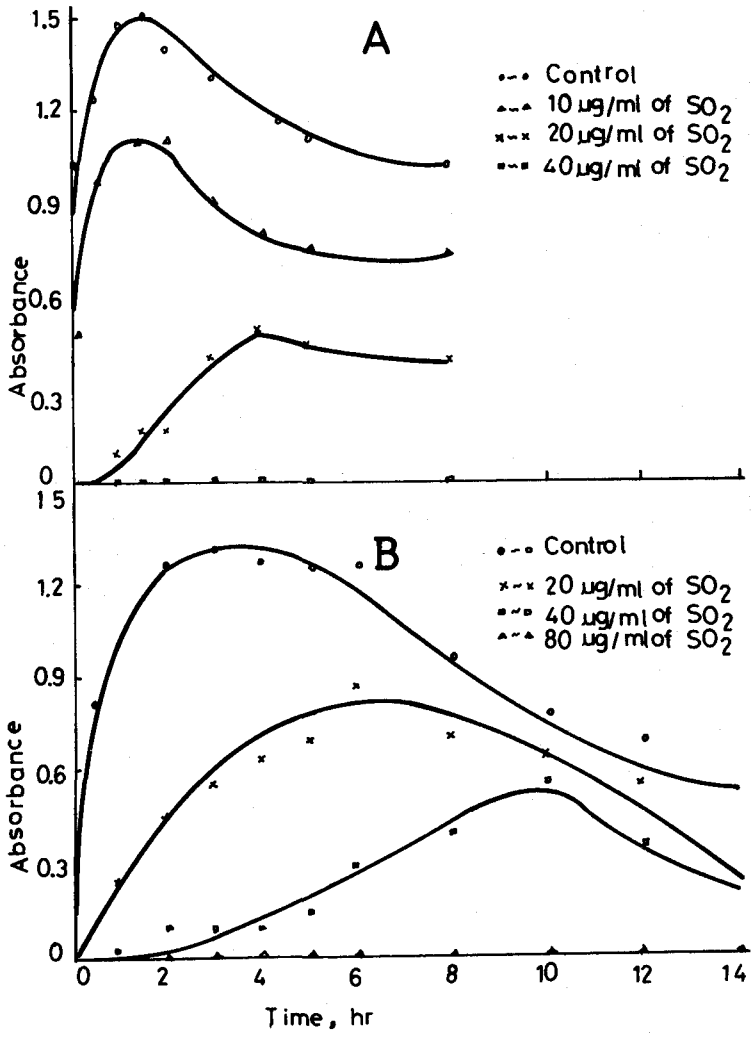

Fig. 4. The pattern of inhibition of dopachrome formation as influenced by various concentrations of SO_2 added at the initiation of reaction in the presence of enzyme preparation from homogenized tissues (A) or sliced potato disks (B). The pH of the SO_2 solution was 6.0 and the final concentration of DOPA was 6 mM. Points are means of four replications.

in the presence of crude enzyme preparation or sliced potato disks. Bedrosian et al[12] reported that the optimum pH of SO_2 was in the range of pH 4–5 when it was used as the sole inhibitor for enzymatic browning in apple tissues. At a pH value higher than 5, apple slices were extremely poor in color.

The distribution of various ionic species of sulfurous acid at different pH values was shown by Vas and Ingram[13]. Possible ionic species in the range of pH 0–8 include undissociated sulfurous acid, bisulfite ion and sulfite ion. In the present study, the great

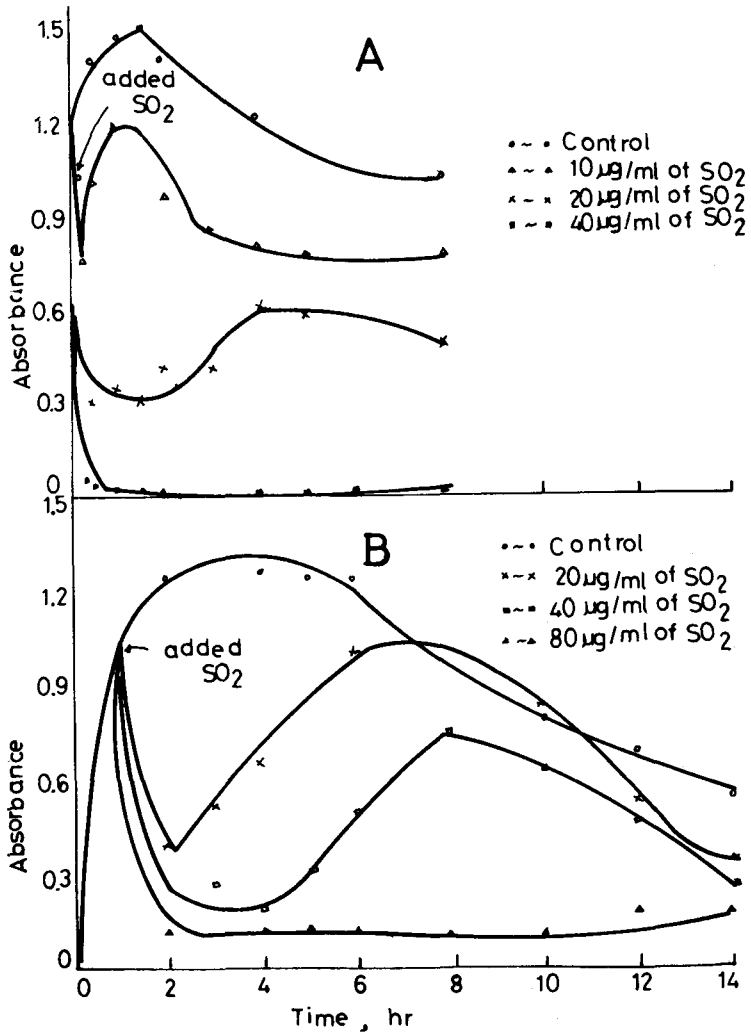

Fig. 5. The pattern of inhibition of dopachrome formation as influenced by various concentrations of SO_2 added 10 minutes after dopachrome formation in the presence of enzyme preparation from homogenized tissues (A), and one hour after dopachrome formation in the presence of identical number of disks (B). pH of employed SO_2 solution was 6.0. and final concentration of DOPA was 6 mM. Points are means of four replications.

differences in dopachrome formation between the control containing no SO_2 and various pH's of SO_2 solution suggest that all of ionic species affect dopachrome inhibition.

Fig.4 shows the pattern of inhibition of dopachrome formation by various concentrations of SO_2 added at the initiation of reaction. The reaction was allowed to proceed for 8 and 14 hours respectively in the presence of crude enzyme preparation and sliced potato disks. The pattern of dopachrome formation at 10 μg/ml of SO_2 showed a shape similar to that of the control. Even 10 μg/ml of SO_2 markedly inhibited the dopachrome formation. The dopachrome formation was greatly suppressed at 20 μg/ml SO_2 and exhibited a short lag period. The dopachrome did not appear within 8 hours after the addition of 40 μg/ml of SO_2. Since the degree of dopachrome formation inhibited by SO_2 did not recover to the level of the control during the entire course of this experiment, it does not appear that SO_2 acts simply by reducing oxygen and making it unavailable for oxidation[2]. The degree of dopachrome formation should recover to the level of the control when the SO_2 is completely used up if SO_2 acted simply by reducing oxygen. The pattern of dopachrome inhibition clearly shows the the required concentration of SO_2 in disks is approximately twice as much as that in the crude enzyme preparation.

The inhibiting pattern of dopachrome formation by various concentrations of SO_2 added after the dopachrome was already formed is shown in Fig. 5. SO_2 was added 10 minutes after the initiation of the reaction in the presence of crude enzyme preparation and one hour after in sliced potato disks. The reactions were allowed to proceed for 8 and 14 hours, respectively.

The optical density dropped immediately after adding all concentrations of SO_2. After a lag period, the dopachrome formation was reinitiated at the lower concentrations of SO_2. However, the degree of dopachrome formation generally did not recover to the level of control. If SO_2 only inactivated the enzyme, the dopachrome would be increased no further and would not show the sudden reduction of optical density. Therefore, it is more probable that SO_2 acts as a bleaching agent in this reaction. SO_2 may not reduce dopachrome to the preceding leucocompound or other intermediates since the degree of dopachrome formation does not recover to the level of control. According to Embs and Markakis[5], SO_2 forms addition compounds with o-benzoquinone, an intermediate of enzymatic browning, and prevents the condensation of the o-benzoquinone to delay the enzymatic browning.

It seems that intermediate products produced in enzymatic browning were combined with SO_2 and produced colorless compounds until SO_2 was no longer available. When SO_2 added in the reaction mixture was used up completely, the dopachrome formation was reinitiated However, the degree of dopachrome formation did not reach to the level of the control since a part of the substrate was already consumed.

국 문 초 록

감자의 마쇄된 조직에서 추출한 효소액과 감자박편을 이용하여 효소적 변색에 대한 SO_2의 억제 기작을 조사하였다. SO_2는 경쟁적인 방식으로 dopachrome형성을 억제하는 것으로 나타났다. 불충

분한 SO_2의 농도에서는 시간이 경과함에 따라 dopachrome의 형성이 재개시되었다.

본 실험의 결과는 SO_2가 효소적 갈변중의 중간산물과 addition compounds를 형성하여 효소적 변색을 지연시킬 수도 있다는 것을 시사하였다. 감자박편에서 dopachrome생성을 억제하기 위하여는 마쇄된 조직에서보다 더 많은 SO_2가 요구되었다.

ACKNOWLEDGMENT

The authors wish to express their sincere appreciation to Dr. E.E. Ewing, Dept. of Vegetable Crops, Cornell University, for thorough reading and valuable corrections of this manuscript.

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