Incidence de la métaplasie épidermoïde

Milieu de culture	Pourcentage d'explants
199 (Earle) non-enrichi	2.8
199 (Earle) + insuline 5 µg/ml	25.6
199 (Earle) + glucose 5 mg/ml	17.6
199 (Earle) + insuline 5 µg/ml + glucose 5 mg/ml	14.3

rence principale entre ces 2 préparations concerne leur concentration de $NaHCO_3$, qui est respectivement de 2,2 et 0,35 g/l. Certains additifs ont été ajoutés au milieu 199 de base, en l'occurence de l'insuline (5 µg/ml) et/ou du glucose (concentration finale de 5 mg/ml). Les cultures ont été faites par immersion. Des prélèvements ont été effectués chaque jour au cours d'une période de 25 jours. Le milieu de culture était renouvellé tous les 3 jours.

Résultats. La métaplasie pavimenteuse de l'épithélium mammaire n'a été observée que lors de l'utilisation du milieu 199 préparé selon la formule de Earle ($NaHCO_3$: 2,2 g/l). L'incidence globale du phénomène atteignait 15,5 ± 5,7% (DS) des explants. Son apparition la plus précoce fut notée dès le 4e jour de culture; en moyenne la date d'apparition la plus fréquente était située aux environs du 12e jour. L'influence des additifs au milieu (tableau) montre que l'apport d'insuline et/ou de glucose favorise de façon hautement significative l'apparition de la métaplasie par rapport aux conditions basales en milieu non-enrichi. Les pourcentages entre les 3 milieux enrichis ne diffèrent pas de façon statistiquement significative.

Discussion. La glande mammaire humaine explantée n'est pas la seule à présenter des phénomènes de métaplasie; la glande de rongeur réagit de cette façon en présence de 7,12-dimethylbenz(a)anthracène[5]. Dans l'épithélium bronchique explanté le déficit en vitamine A est un facteur étiologique[6]; l'addition de vitamine A empêche le phénomène[7]. Nous pensons que ce facteur n'intervient pas dans la glande mammaire puisque le milieu 199 contient 0,1 mg/l de vitamine A; or cette concentration suffit à empêcher la métaplasie dans les bronches[7].

Si nos observations permettent de penser que le pH joue un rôle, il faut toutefois envisager l'influence des mécanismes de réparation dont la métaplasie pavimenteuse est considérée comme le témoin[8]. La métaplasie et la kératinisation impliquent un taux de mitoses suffisant pour produire assez de cellules capaples de constituer un sytème de formation de kératine[9]. Dans la trachée la synthèse d'ADN est notablement accrue dans les foyers de métaplasie en culture. Il est actuellement bien établi que l'insuline joue un rôle dans la synthèse d'ADN et la division cellulaire des tissus en culture. Nos observations confirment le rôle stimulant de l'enrichissement insulinique et/ou glucosé sur l'apparition de la métaplasie dans l'épithélium mammaire explanté. Toutefois, le pH du milieu constitue un facteur déterminant puisque dans les mêmes conditions d'enrichissement insulino-glucosé la métaplasie n'apparaît jamais en milieu 199 de Earle.

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Effect of indole-3-acetic acid on the kinetics of horseradish peroxidase catalyzed scopoletin oxidation

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16 March 1977

Summary. The kinetics of horseradish peroxidase catalyzed scopoletin oxidation were observed to be sigmoidal. The apparent K_ms for scopoletin is 0.9 mM. Inhibition of scopoletin oxidation by indole-3-acetic acid (IAA) appears to be non competitive. Non competitive inhibition by IAA suggests a more significant role of the peroxidase protein matrix in regulating the activity of the heme moiety.

The interest in physiological substrates for peroxidase includes scopoletin, a naturally-occurring coumarin which has been shown to inhibit growth in tobacco seedlings[2]. Although Andreae[3] first demonstrated scopoletin oxidation by crude IAA oxidase preparations of potato tissue in 1952, it was the work of Imbert and Wilson[4] on the modulation of growth hormone degrading activity (IAA oxidase) by scopoletin that has led to several studies of the interaction of scopoletin and/or IAA with peroxidases. Sirois and Miller[5] postulated mechanisms to explain the non-linear competitive nature of inhibition of IAA oxidase activity by scopoletin and demonstrated the oxidation of scopoletin in the presence of IAA by horseradish peroxidase. Sigmoidal kinetics were shown for the oxidation of scopoletin in the absence of IAA by tobacco tissue culture isoperoxidase A_3[6], but after more extensive investigation, this enzyme was shown to contain only a single subunit, although many of the characteristics of substrate cooperative effects or 'allosterism' were observed[7]. The kinetics of scopoletin oxidation by horseradish peroxidase were studied and the effects of IAA upon these kinetics were evaluated to probe the mecha-

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nistic nature of peroxidase/IAA/scopoletin interactions. *Methods and materials.* Horseradish peroxidase (HRP) was obtained from Sigma Chemical Co. HRP type VI had an RZ value of 2.7 and an activity of 290 Sigma purpurogallin units per mg. The concentration of HRP was determined spectrophotometrically, using a molar absorptivity of 9.1×10^4 $M^{-1}cm^{-1}$ at 403 nm[8]. Scopoletin and IAA were obtained from Sigma Chemical Co. Scopoletin was recrystallized from methanol before use. Enzyme assays were run at the pH optimum of 4.5 and

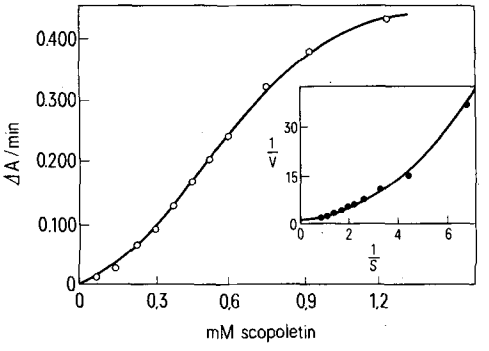

Fig. 1. Effect of scopoletin concentration on the velocity of HRP-catalyzed scopoletin oxidation. Assays were run at 5 mM H_2O_2 and 9×10^{-11} M HRP in 40 mM sodium citrate buffer (pH 4.5). Inset: Double reciprocal plot.

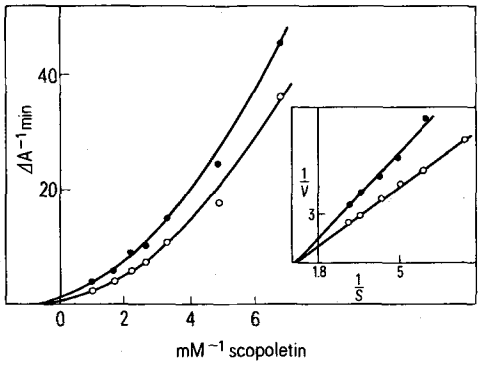

Fig. 2. Effect of IAA on the double reciprocal dependence on scopoletin concentration of HRP catalyzed scopoletin oxidation. Assays were run at 5 mM H_2O_2 and 9×10^{-11} M HRP in 40 mM sodium citrate buffer (pH 4.5). -○-○-, No. IAA; -●-●-, 0.0225 mM IAA. Inset: 1/velocity versus 1/[scopoletin]$^{1.8}$ for same curves.

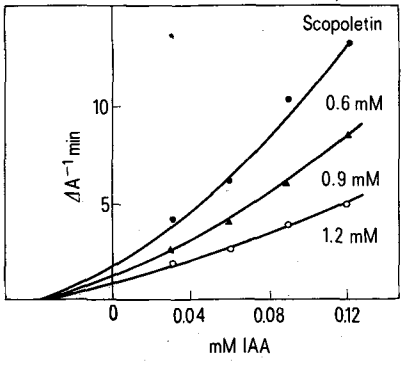

Fig. 3. Effect IAA concentration on the reciprocal velocity of scopoletin oxidation at various scopoletin concentrations (1/V versus 1). Assays were run at 5 mM H_2O_2 and 9×10^{-11} M HRP in 40 mM, sodium citrate buffer (pH 4.5). -●-●-, 0.6 mM scopoletin; -▲-▲-, 0.9 mM scopoletin; -○-○-, 1.2 mM scopoletin.

contained scopoletin, 5 mM H_2O_2, 40 mM sodium citrate, and 9×10^{-11} M HRP. Activity was linear over the range of 1.5×10^{-11} M to 2×10^{-10} M HRP. Assays were run at 23 °C and were initiated by adding enzyme. After a 1–2 min lag phase linear changes in absorbance at 450 nm were obtained.

Results. Figure 1 shows the dependence of velocity on scopoletin concentration for horseradish peroxidase catalyzed scopoletin oxidation. Sigmoidicity is readily confirmed from the concave upward Lineweaver-Burk plot[9] (inset figure 1) and from the Hill plot[10] of this data which yields a slope of $n = 1.8$. These findings are in agreement with the observations for tobacco tissue culture isoperoxidase A_3 catalyzed scopoletin oxidation. The $S_{0.5}$ for scopoletin is 0.9 mM.

Since it was demonstrated that the addition of scopoletin caused sigmoidicity to occur in IAA saturation curves for IAA oxidase activity of horseradish peroxidase[5], the effect of IAA on scopoletin oxidation by HRP was determined. Figure 2 shows the effect of IAA on the Lineweaver-Burk plots of scopoletin saturation curves. Inhibition is observed and both curves are concave upward implying sigmoidicity in the saturation curves. These curves can be made linear by a plot of 1/V versus $1/S^n$ where n is the Hill slope, $n - 1.8$ for scopoletin saturation in the absence of IAA (inset figure 2). A least squares analysis of the linear plot yields 1/V intercepts of 1.34 ± 0.08 and 1.80 ± 0.22 for the plots without and with IAA respectively. $1/S^{1.8}$ intercepts are 1.24 ± 0.17 for the plot without IAA and 1.22 ± 0.19 with IAA present. Figure 3 shows the 1/V versus I plot for IAA inhibition of scopoletin oxidation. The nonlinearity of the curves in figure 2 makes extrapolation difficult. The linear plot (inset figure 2), however, indicates that intersection occurs on or near the $1/S^{1.8}$ axis suggesting noncompetitive or mixed inhibition with a predominance of the noncompetitive mode[11]. This observation is also apparent for figure 3, even though the plots are not linear.

Discussion. Since horseradish peroxidase is known to contain only a single subunit with one heme group per unit, it is apparent that sigmoidal kinetics cannot be explained by classical subunit interaction. Although competitive inhibition might be expected for a single subunit protein such as HRP, noncompetitive or mixed inhibition is consistent with the observations of Galston et al.[12], who proposed that peroxidation and IAA oxidation take place at different sites on HRP. Dissociation of heme from the holoenzyme did not affect IAA oxidation by the apoenzyme but eliminated guaiacol oxidizing activity. IAA might then exert an allosteric (classical noncompetitive) effect by binding at a site removed from the site of scopoletin oxidation. The nonlinear competitive effects of scopoletin on IAA oxidation observed by Sirois and Miller[5] could be explained by binding of scopoletin at both the peroxidase and oxidase sites and IAA binding at only the oxidase site. The complexity of the peroxidase mechanism could lead to many kinetic patterns, however, present observations suggest that peroxidase structural consideration may possibly be more complex than that of rigid heme active site dominating a noninteracting protein matrix.

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