Stimulation of Induction of Glucose Dehydrogenase by Homologous Series of Aliphatic Alcohols in Dormant Spores of *Aspergillus oryzae*

When suspended in water, conidia of the rice mold, *Aspergillus oryzae*, neither germinate nor swell for months[1]. During this period, however, some enzymes are inducible[2], and the rate of induction is enhanced by some alcohols mainly at the stage of translation, possibly by facilitating the formation of initiation complex[3]. The present paper extends this study to include homologous series of aliphatic alcohols.

The conidia were produced, harvested, and freed from mycelial fragments as described previously[2]. Glucose dehydrogenase was induced and assayed as described previously[2,4].

Figure 1 shows that lower concentrations of various alcohols stimulated the rate of induction, whereas higher concentrations were inhibitory. Methanol and ethanol had no stimulatory effect at any concentrations tested (not shown in Figure 1). For convenience of analyzing the data, the maximal alcohol concentration that caused neither stimulation nor inhibition was referred to as C_m, and was obtained from Figure 1 where concentration-response curves intersect the relative rate of 1.0. C_m decreased with increasing the hydrocarbon chain length of the alcohols, while C_m increased with branching in the chain. In Figure 2, log C_m is plotted against the boiling point and against the chain length of a series of *n*-alcohols. In both cases, a linear correlation between 2 parameters was observed. These results would suggest that hydrophobic interactions were responsible for the stimulatory effect of the alcohols, since it is known[5] that the hydrophobicity of aliphatic alcohols is reflected by some physical-chemical constants, such as boiling point and solubility in water, and that the contribution of each CH_2 group to hydrophobic bonding is additive. The standard free energy change of interaction for each CH_2 group of the alcohols, $\Delta G°$, was calculated from the least-square slope of the line in Figure 2b by the use of equation[6]

$$\Delta G° = R\,T\,1n\,C_{i+1}/C_i$$

where C_i and C_{i+1} refer to the C_m values of *i*th and *i*th + 1 homologues, respectively; R and T are the gas constant and the absolute temperature, respectively. The value obtained was −650 cal/mole. It was reported[6] that in several model systems, free energy transfer from an aqueous phase to completely nonpolar phase, such as triolein or light petroleum, is between −750 and −883 cal/CH_2 group. The lower value obtained in the present system may indicate the incomplete dehydration of CH_2 groups when the alcohols were adsorbed to the receptor site. This suggests that the receptor site is less hydrophobic than the nonpolar phases in the model systems. ROTH and SEEMAN[7] showed that biological membranes were much less hydrophobic than 1-octanol, since membrane/buffer partition coefficients for several aliphatic alcohols were invariably $^1/_5$ of 1-octanol/water partition coefficients.

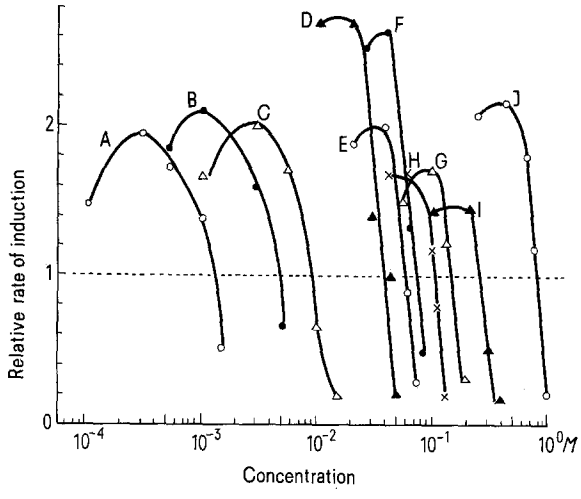

Fig. 1. Effect of alcohols on the rates of induction of glucose dehydrogenase. The rate in the absence of alcohol is represented by the value of 1.0 on the ordinate. A) refers to 1-octanol; B) 1-heptanol; C) 1-hexanol; D) 1-pentanol; E) isopentanol; F) *sec*-amyl alcohol; G) *tert*-amyl alcohol; H) 1-butanol; I) 1-propanol; and J) isopropanol.

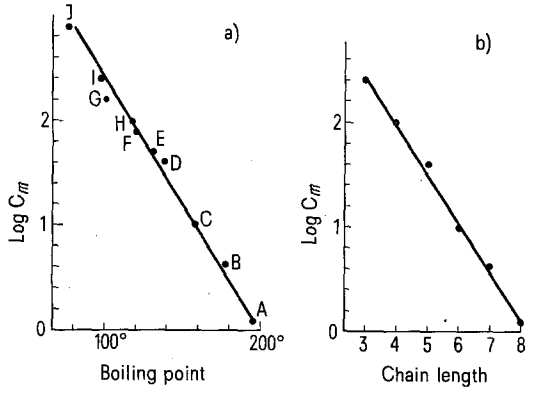

Fig. 2. a) Relationship between log C_m and boiling point of alcohols. The Roman letter besides each dot stands for the same alcohols as in the legend to Figure 1. C_m is defined as described in the text, and is expressed as m*M*. b) Relationship between log C_m and the number of carbon atoms in a homologous series of *n*-alcohols.

Aliphatic alcohols were shown to cause a variety of effects on many types of cells; e.g., protection of erythrocyte from hypotonic hemolysis[7], paramecium immobilization[8], inhibition of muscle contraction[9], and narcosis of various animals[6,9]. Although the precise mechanism underlying these phenomena is not clear at present, cellular membranes were suggested to be the primary site of action in all cases so far examined. SEEMAN et al.[10] showed that homologous alcohols at concentrations similar to those of C_m expanded the membrane area of intact erythrocyte by approximately 3%. If one assumes that the alcohols caused a similar membrane expansion in the dormant conidia, it is compatible with 'membrane brake' hypothesis[3] that the low activity of protein synthesis in dormant conidia is due to the structure of

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endoplasmic reticulum membrane, which acts as a partial brake by limiting the formation of initiation complex. Possibly the membrane is kept in a condensed state and the site where the ribosome is attached is partially buried. On binding the alcohols, the membrane may undergo a conformational change, e.g. an expansion, and the ribosome-attaching site may be more exposed. This may result in the stimulation of formation of initiation complexes for proteins that are synthesized by membrane-bound ribosomes. Although further work is needed to substantiate this hypothesis, it is tempting to speculate that the dormancy of fungal spore may be maintained, at least in part, by this 'membrane brake' mechanism.

Résumé. La mise en action de la glucose déshydrogénase dans la spore dormante d'*Aspergillus oryzae* est stimulée par l'addition de séries homologues des alcools aliphatiques. La concentration de l'alcool nécessaire à cet effet diminue avec l'accroissement de longeur de la chaîne hydrocarbonée, mais augmente avec la ramification de la chaîne.

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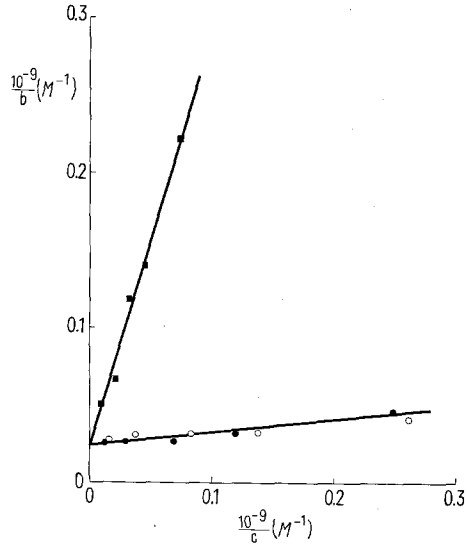
14 June 1972.

Dissociation Constants of the Complexes between RNA-Polymerase II and Amanitins

Amanitins, the main toxins of *Amanita phalloides*[1,2], inhibit eukariotic RNA-polymerase II by binding to the enzyme[3–8]. In the present experiments the K_d of the complexes of the enzyme with methyl-γ-amanitin, with α-amanitin and with the non-toxic derivative methyl-aldoamanitin were investigated by equilibrium dialysis.

RNA-polymerase II was obtained from rat liver according to ROEDER and RUTTER[9] and was stored at −195° in TGMED (0.05 *M tris*-HCl, pH 7.9, 25% (v/v) glycerol, 5 m*M* $MgCl_2$, 0.1 m*M* EDTA, 0.5 m*M* dithiotreitol) containing 0.1 *M* $(NH_4)_2SO_4$. Activity was measured[5] on 0.2 ml of the enzyme preparation using calf thymus heat-denaturated DNA as template and ^{14}C-ATP (The Radiochemical Centre, Amersham, Great Britain; 2 mCi/mmol) as labelled nucleotide. 610 pmoles of AMP were incorporated in the perchloric acid insoluble material after a 10-min period at 37 °C.



Binding of methyl-γ-amanitin to rat liver RNA-polymerase II in the absence (●) and in the presence of α-amanitin (■) or of methyl-aldoamanitin (○). Plot of 1/b against 1/c from equilibrium dialysis of increasing concentrations of ^{14}C-methyl-γ-amanitin (6–60 n*M*) in the presence of a constant amount of RNA-polymerase II. The equation for the straight line, calculated by the least-squares method, was $1/b = 0.023 \times 10^9 + 0.083/c$. The equation was not significantly modified in the presence of a constant concentration (100 n*M*) of methyl-aldoamanitin ($1/b = 0.023 \times 10^9 + 0.072/c$), while in the presence of α-amanitin (100 n*M*) it was $1/b = 0.023 \times 10^9 + 2.69/c$.

Equilibrium dialysis experiments were performed in Plexiglas chambers separated by cellophane disks cut from a Visking 20/32 tube (Visking Co., Chicago, Ill., USA), previously stretched according to CRAIG and KING[10] in order to increase the porosity of the membrane and the dialysis rate of amanitins. A 35% increase of both diameter and length of the tube during the stretching allowed equilibrium of ^{14}C-methyl-γ-amanitin (68 mCi/mmol) through the membrane to be reached in 20 h at 4 °C in TGMED. 0.2 ml of the enzyme solution were placed in one compartment of the dialysis chambers and 0.2 ml of TGMED containing 0.1 *M* $(NH_4)_2SO_4$ in the other. The final volume in both compartments was brought to 0.25 ml by addition of the ligands. ^{14}C-methyl-γ-amanitin was initially present in the same amount in both compartments, in the range from 1.5 to 15 pmoles. Duplicates were also set up containing on both sides of the dialysis membrane a constant amount (25 pmoles) of non-radioactive α-amanitin or of non-radioactive methyl-aldoamanitin. After continuous shaking for 24 h at 4°C, two 0.1 ml aliquots were withdrawn from each compartment and radioactivity was measured in a Nuclear-Chicago mark I scintillation counter. The counts were corrected to 100% efficiency. Free ^{14}C-methyl-γ-amanitin concentration was calculated from the radioactivity of the compartment without the enzyme. Bound ^{14}C-methyl-γ-amanitin was calculated by difference between the radioactivities of the two compartments,

Results are plotted in the Figure according to the equation[11] $1/b = 1/a + K_d/ac$, where b and c are the concentrations of bound and free ^{14}C-methyl-γ-amanitin, a is the molarity of the enzyme binding sites, and K_d the dissociation constant. The plot gives a straight line in which the intercept of the ordinate ($1/a$) allows the calculation of the molarity of binding sites and the

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