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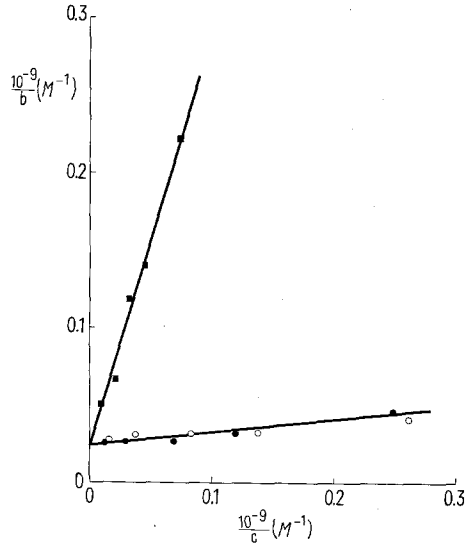
Dissociation Constants of the Complexes between RNA-Polymerase II and Amanitins

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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intercept of the abscissa is $-1/K_d$. In the presence of a competitive ligand the plot has the same intercept on the ordinate axis, but the slope increases by a factor equal to $(1 + [I]/K_i)$ where [I] is the concentration and K_i the dissociation constant of the competitive ligand[12]. The results reported in the Figure indicate a molarity of binding sites of 43×10^{-9}. The K_d of the complex of methyl-γ-amanitin with RNA-polymerase II was $3.6 \times 10^{-9} M$. The presence of 100 nM α-amanitin inhibited in a competitive way the binding of ^{14}C-methyl-γ-amanitin and the K_i for α-amanitin, calculated from the ratio between the two slopes of the Figure, was $3.2 \times 10^{-9} M$. These results indicate that the affinities of amanitins for RNA-polymerase II are very high, the dissociation constants being of the same order as those reported for the strongest binding between haptens and their specific antibodies (see[13].) The Figure shows that the presence of 100 nM methyl-aldoamanitin does not interfere with the binding of ^{14}C-methyl-γ-amanitin. The non-toxic methyl-aldoamanitin lacks the γ-OH group present in the isoleucine side chain of both methyl-γ-amanitin and α-amanitin[14]. It does not inhibit RNA-polymerase[15] and our results indicate that it does not bind to the enzyme[16].

Riassunto. Sono state calcolate le costanti di dissociazione dei complessi fra la RNA-polimerasi II e la metil-γ-amanitina, la α-amanitina e la metil-aldoamanitina. La K_d è risultata essere $3.6 \times 10^{-9} M$ per la metil-γ-amanitina e $3.2 \times 10^{-9} M$ per la α-amanitina. La metil-aldoamanitina non si lega all'enzima.

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Free Amino-Acids Composition of Larvae and Pupae of *Euproctis fraterna* Moore (Lepidoptera: Lymantriidae) Reared on the Leaves of Different Host Plants

The *Euproctis fraterna* Moore is commonly known as castor hairy caterpillar. The larvae are polyphagous and attack a variety of host plants e.g., plum, apple, mango, apricot, peach, pear, grapevines, citrus, rose, castor and cotton[1]. Recently, some attention has been focussed on the nutritional studies of insects and insect-plant relationship which partly reflect the capacity of the insect to utilize the various constituents of hosts including amino acids and proteins[2-4]. Since very little is known about the physiology of *Euproctis fraterna*, the present investigations were undertaken to study the changes in free amino acids composition of larvae and pupae reared on the leaves of different host plants.

Material and methods. The larvae were reared on the leaves of 3 host plants viz., castor bean (*Ricinus communis*), ber (*Ziziphus mauritiana*) and crab-apple (*Malus hupehensis*) under the laboratory conditions. Before crushing the larvae in 80% alcohol for amino acid analysis, they were starved for 24 h. The larvae were analyzed at the 5th or 6th instar, whereas the pupae were taken at the full grown stage. The larvae and pupae were washed with distilled water, then dried on blotting paper and weighed. 20 insects were taken for analysis and there were three replicates in each treatment. The alcoholic extract of the insect was made and the free amino acids were analyzed by the procedure of PAL and LALORAYA[5] employing two dimensional ascending paper chromatography. Whatman No. 1 filter paper was used in the present investigation. Phenol (80% in double distilled water) saturated with 0.5% ammonia solution was used as the first running solvent, whereas *n*-butanol, acetic acid and water (4:1:5) was the second solvent. The dried chromatograms were sprayed with 0.1% ninhydrin and the various amino acid spots were developed by heating the chromatograms in an oven at 80 °C for 30 min. The amino acids were identified on the basis of their Rf values and colour reactions in different solvent systems. The quantitative estimation of amino acids was done by the colorimetric method. The data are expressed in terms of glycine.

Results. The Figure shows the growth rates of larvae of *Euproctis fraterna* reared on the leaves of different host plants. It is clear that larvae on the castor leaves comparatively develop faster than on ber and crab-apple. Furthermore, the weights of the larvae and pupae on castor leaves are more than on ber and crab-apple. This may be due to host preference of this pest to castor as compared to ber and crab-apple.

The alcoholic extracts of larvae and pupae of *Euproctis fraterna* contain about 18 amino acids (Table). These are leucines and phenylalanine, valine, γ-aminobutyric acid, tyrosine, proline, α-alanine, glutamic acid, threonine, arginine, aspartic acid, glycine and serine, asparagine, glutamine, histidine and lysine and 2 unidentified spots numbered 'x' and 'y'. It is clear from the Table that the amino acids composition of the larvae and pupae reared on the leaves of different host plants, shows marked differences in their relative concentration. It has been observed that larvae reared on castor bean leaves show, in general, more amino acids as compared to ber and crab-apple. Leucines and phenylalanine, valine, proline, glutamic acid, glycine and serine show highest concentration in larvae reared on castor bean leaves; whereas γ-aminobutyric acid and threonine show maximum concentration in larvae reared on crab-apple. However, tyrosine, α-alanine, arginine, aspartic acid, asparagine, glutamic acid, histidine and lysine

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