(log $R_m$) versus gel concentration for the 2 bands of enterotoxin $C_2$ (Figure 1) gave parallel lines (Figure 2). The regression lines were calculated on a SCM Marchant desk calculator 1016 PR. Parallel lines show that the 2 bands have the same size and therefore exhibit the same retardation coefficient, but differ in charge[3].

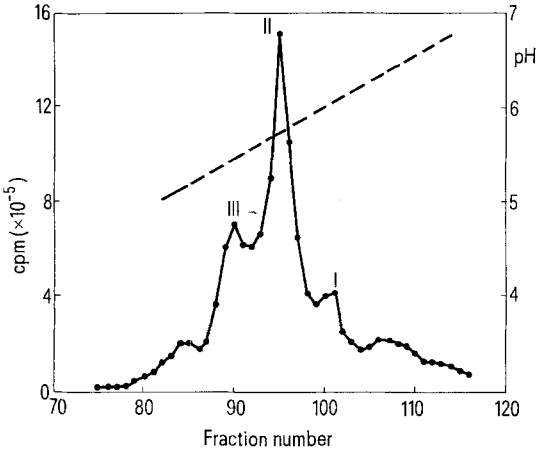


Fig. 3. Isoelectric focusing of $^{125}$I-enterotoxin $C_2$ (46 $\mu$Ci/$\mu$g; 0.6 atom/molecule) using LKB carrier ampholytes with isoelectric point values between pH 3 and 10. 140 3.0-ml fractions were collected and analyzed for radioactivity (●) in a $\gamma$-ray spectrometer, and for pH (broken line) at room temperature.

Binding of various fractions obtained by isoelectric focusing of $^{125}$I-enterotoxin $C_2$ to enterotoxin $C_2$ antibody

| Components | pI | Counts per min[a] added | Counts per min bound (%) |
|---|---|---|---|
| I | 6.13 | 3,342 | 37.5 |
| | | 8,719 | 28.5 |
| II | 5.73 | 15,658 | 15.7 |
| | | 38.796 | 12.3 |
| III | 5.45 | 18,100 | 17.3 |
| | | 38,370 | 13.1 |
| Unfractionated | | 9,016 | 18.7 |
| | | 33,480 | 10.7 |

[a] A portion from each of the fractions (I, II, III, of Figure 3) containing peak radioactivity was added to antibody-coated polystyrene tubes.

The heterogeneity of enterotoxin $C_2$ was corroborated by isoelectric focusing[9]. The toxin can be trace-labelled with radioactive iodine thereby providing for sensitive detection of enterotoxin in the electrofocusing pattern. As shown in Figure 3, a sharp peak focused at pH 5.73, among several other peaks in a pH range between 5.0 and 7.0. Each of the fractions tested showed good binding to solid-phase antibody, as compared with unfractionated enterotoxin $C_2$ (Table). Also, each isoelectric component of enterotoxin $C_2$ (peaks I, II, and III shown in Figure 3) was chromatographed in a Sephadex G-75 column, and eluted with the same $K_{av}$ (0.37) as unfractionated enterotoxin $C_2$, indicating that enterotoxin $C_2$ had not formed stable aggregates or immunologically active fragments during electrofocusing. In 6 *M* urea-0.01 *M* dithiothreitol an essentially similar isoelectric spectrum was obtained (figure not shown). When a correction[6] was made for the effect of 6 *M* urea on measured pH, no variation in isoelectric point due to the presence of 6 *M* urea was observed with labelled toxin. Therefore, the isoelectric heterogeneity of enterotoxin $C_2$ does not result from conformation-dependent variations in the dissociation of acidic and basic side chains of the protein molecule. It seems likely that these differences occur due to loss of particular amide groups in the protein as has been demonstrated for enterotoxin B (note cited in[10,11]) and $C_1$ (L. Spero, private communication cited in[12]). See also Williamson et al.[13].

*Résumé.* L'hétérogénéité de l'entérotoxine $C_2$ d'un staphylocoque par électrophorèse en gel polyacrylamide est reliée à la présence d'isomers de charge différente.

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## Studies on the Adaptive Nature of Cellulolytic Enzyme from *Chaetomium aureum* Chivers

A few microorganisms[1,2] have been reported to produce cellulolytic enzyme adaptively; but information on the nature of cellulase produced by *Chaetomium* spp. is still lacking. *Chaetomium aureum* was therefore tested for the nature of cellulase it produces with a view to exploring the possibility of using the cellulolytic character as a marker in genetic investigations[3]. The culture medium used in the experiment was modified Czapek-Dox[4] adjusted to pH 6.5 before sterilization. Sterilization was done at 10 lbs pressure for 20 min. Glucose, cellulose and combination of cellulose and glucose, in the proportion of 1.5% each, were used as the carbon source. The medium having only the inorganic chemicals was considered as the control medium. Determination of cellulase activity was done qualitatively by depth of clearing[5] and quantitatively by estimation of reducing sugars[6–8]. For qualitative test stab was made with 10 ml of solid medium having 1.5% purified agar[9] in each culture

Effect of carbohydrate source on cellulase activity of *C. aureum*

| Medium[a] | Cellulase activity | |
|---|---|---|
| | Depth of clearing (mm) | N/200 $Na_2S_2O_3$ solution (ml) |
| Medium + glucose | — | — |
| Medium + glucose + cellulose | — | — |
| Medium + cellulose | 5.92 | 14.39 |
| Medium control | — | — |

[a] Modified Czapek-Dox; -absence of activity.

tube (150 × 14 mm). For isolation and estimation of cellulase, *C. aureum* was grown in 75 ml of broth in 250 ml Erlenmeyer flasks.

Cellulose pulp[10] and filter paper (Whatman No. 1) were used as the cellulose source for stab and broth respectively. 0.7 mm diameter of inoculum disc of *C. aureum* grown on modified Czapek-Dox medium having cellulose pulp[10] as the only carbon source, and 0.5 ml of ascospores suspension ($12.75 \times 10^6$), were used for stab and culture broth respectively. Incubation was done at 30 °C for 10 days. 5 replicates were considered for each observation.

It is evident from the Table that induction of cellulolytic enzyme was noticed only in cellulose supplemented medium. But in no case did glucose, and cellulose in combination with glucose, induce cellulase activity. This observation confirms that cellulase of *C. aureum* is not a constitutive enzyme but an adaptive one, since it is elaborated only in response to a specific substrate i.e., cellulose, as was observed in other organisms[1,2].

*Zusammenfassung.* Das cellulolytische Enzymsystem von *Chaetomium aureum* (Chivers) ist adaptiv. Seine Aktivität wird durch Cellulose, nicht aber durch Glukose oder Glukose plus Cellulose induziert.

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## Effect of Allicin on Certain Enzymes of Liver After a Short Term Feeding to Normal Rats

In a recent paper[1], the authors have reported the lipid lowering effect of allicin on long-term feeding to normal rats. The authors have found also that allicin significantly lowered the blood sugar of alloxan diabetic rabbits, and that its effect was similar to that of tolbutamide[2]. The present paper deals with the effect of allicin on certain enzymes of liver after a short-term feeding of the drug to normal rats.

*Material and methods.* Four-month-old, young male wistar rats of average weight (125 g) were divided into 2 groups of 6 animals each. They were fed on normal laboratory diet as reported earlier[1]. Allicin was prepared from fresh garlic cloves, according to the method of CAVALLITO and BAILEY[3] for the present study. The initial fasting blood sugar level of all the rats was determined by the method of ASATOOR and KING[4]. Blood was collected from the tail. One group was kept as control and to the other group freshly prepared allicin (Dose 100 mg/kg/day) was orally administered as a solution in 2 ml distilled water. After 15 days treatment, the fasting (18 h) blood sugar was again determined as before in both the groups. Then they were sacrified by decapitation. Certain enzymes of the liver viz. hexokinase, α-glucan phosphorylase, glucose-6-phosphatase and lipase were determined by standard methods. Liver tissue was used for the enzyme preparation as reported from this laboratory[5].

Hexokinase (E.C. 2.7. 1.1. ATP. D-hexose-6-phosphotransferase) activity was determined by the measurement of disapperance of glucose in the presence of ATP[6]. The chilled liver tissue was homogenized at 0 °C with 3 volume of buffer of the following composition. *Tris*-0.1 *M*, histidine 0.1 *M*, EDTA-0.01 *M* and $MgCl_2$ 0.01 *M* (pH 7.0). The homogenate was centrifuged at 3000 × *g* at 0 °C for 5 min and the supernatant was used as the enzyme preparation. Enzyme activity was assayed by the method described by CRANE and SOLS[7]. One unit of enzyme activity is that amount which catalyse the phosphorylation of 1 μmol of glucose in 15 min at 30 °C under otherwise optimal conditions.

α-Glucan phosphorylase (E.C. 2.4.1.1. α-1, 4-glucan or the phosphate glucosyl transferase). Phosphorylase activity was determined by measurement of the rate of liberation of inorganic phosphate from glucose-1-phosphate in the presence of glycogen[8]. i.e. phosphorylase activity was measured in the direction of synthesis of polysaccharide. The chilled liver tissue was homogenized at 0 °C in 3 volume of 0.1 *M* NaF. Centrifuged at 1500 × *g* at 0 °C and the supernatant was used as the enzyme. Enzyme activity was assayed by the method of SUTHERLAND[9]. One unit of enzyme was defined as that amount which caused the liberation of 1.0 mg of inorganic

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