SEPARATION OF DETOXIN COMPLEX AND CHARACTERIZATION OF TWO ACTIVE PRINCIPLES, DETOXIN C₁ AND D₁

Sir:
In the preceding communication¹), we have reported the production, extraction and some biological properties of the detoxin complex, obtained as the metabolites of Streptomyces caesiptus var. detoxicus 7072 GC₁.

This communication is concerned with the separation and characterization of two main principles designated detoxin C₁ and D₁.

Preliminary work by paper and thin-layer chromatography have indicated the existence of a number of similar antagonists with different specific activity. In the earlier stage of the purification, it was studied whether the minor components were real or artifacts.

We found it convenient to use buffered resin chromatography according to the procedure of Moore et al.²) for the separation of detoxin complex into eight groups according to the basicity of the components.

Detoxin complex (5.0 g, 530 u/mg) was chromatographed on a column of Dowex 50 W × 2 (4 x 150 cm) at a flow rate of 150 ml/hour. Before use, the resin was equilibrated with 0.2 M pyridine-acetic acid buffer at pH 5.0 (161 ml of pyridine and 160 ml of acetic acid diluted to 10 liters with distilled water), and the sample dissolved in the same buffer was applied. Elution was started with the same buffer (30 liters), and then continued with 0.3 M pyridine-acetic acid buffer at pH 5.0 (241 ml of pyridine and 200 ml of acetic acid diluted to 10 liters with distilled water). Eluate samples of 20 ml were collected mechanically and assayed for antagonism to the inhibitory action of blasticidin S against B. cereus.

Under these conditions, eight distinct active peaks were observed in the eluate.

Appropriate fractions under each peak were combined, concentrated under reduced pressure at 40°C and finally lyophilized to yield detoxins which were designated A, B, C, D, E, F, G, H groups in the order of elution. Each of eight groups thus obtained was examined by paper and thin-layer chromatography and the results suggested that each was still a mixture contaminated with some impurities. The results of resin column chromatography are summarized in Table 1, which suggests that groups C and D might be the main active principles of detoxin complex, because each of these two groups showed higher specific activity than others. Hence further purification was directed to these two groups.

Crude detoxin C group (120 mg; 1,250 u/mg) was separated into detoxin C₁, C₂ and C₃ by partition chromatography on a silica gel column (2 x 20 cm,
Mallineckrodt, 100–200 mesh) using solvent system of butanol–acetic acid–water (3:1:1, v/v). Fractions of 5ml were collected whereupon detoxin $C_1$ was found in tube number 18–21, detoxin $C_2$ in 26–30, and $C_3$ in 35–37. After concentration of the appropriate fractions followed by the lyophilization, detoxin $C_1$ (48mg), detoxin $C_2$ (15mg), and detoxin $C_3$ (7mg) were obtained as amorphous powders. Detoxin $C_2$ and $C_3$ were minor components contaminated with some impurities. Detoxin $C_1$ was further chromatographed on Sephadex G–10 (3×120 cm) with redistilled water to afford a semi-crystalline powder (18mg; 2,200u/mg), the purity of which was confirmed by paper and thin-layer chromatography.

Detoxin $C_1$ recrystallized from water gave microneedles of m.p. 142~144°C, $[\alpha]_{20}^D = -23°$ (c 1, MeOH). The elementary analysis, molecular weight determination by ebullioscopic method and potentiometric titration were consistent with the molecular formula $C_{29}H_{44}O_9N_4$. 

**Fig. 2. Countercurrent distribution pattern of detoxin $D_1$.**

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$\text{mg}$

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$\text{Distribution curve by weight (mg)}$

$\text{Theoretical curve}$

The crude powder of detoxin $D$ group (300mg; 2,500u/mg), although seemingly homogenous by paper and thin-layer chromatography, could be separated into three active components designated detoxin $D_1$ (150mg), $D_2$ (12mg) and $D_3$ (3mg) by gel filtration on a Sephadex G–10 column (3×120 cm). Detoxin $D_1$ was the most important active principle of the detoxin complex with the highest specific activity (5,500u/mg) among the detoxin components. Detoxin $D_2$ and $D_3$, the minor components of $D$ group, were obtained in too low a yield for further characterization.

The homogeneity of detoxin $D_1$ was confirmed by countercurrent distribution (solvent system: n–butanol–0.2 M pyridine–acetic acid buffer, at pH 5.0). The pattern is shown in Fig. 2.

Detoxin $D_1$, m.p. 168°, $[\alpha]_{20}^D = -16°$ (c 1, MeOH), is an amphoteric compound of pKa 8.0 and 4.0 (titration equivalent 610). Anal. Found: C 58.59, H 7.59, O 23.43, N 9.33. Calcd. for $C_{29}H_{44}O_9N_4$:


$C_{30}H_{46}O_9N_4$:

$C 59.39, H 7.64, O 23.73, N 9.24$.

Detoxin $D_1$ is an amphoteric compound with pKa 8.0 and 3.9. UV $\lambda_{\text{max}}$ (E $\%$) 248 (3.70), 253 (3.18), 259 (2.65), 265 (2.80), 269 m$\mu$ (1.85). IR $\nu_{\text{max}}$ 3300, 1740, 1670, 1580, 1250, 1200, 1170, 980 cm$^{-1}$. Attempts to determine the precise molecular formulae of detoxin $C_1$ and $D_1$ by high resolution mass spectrometry were unsuccessful.

Further isolation and characterization of detoxin homologues, together with the structural elucidation of detoxin $C_1$ and $D_1$, are in progress.

$\text{Acknowledgement}$

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